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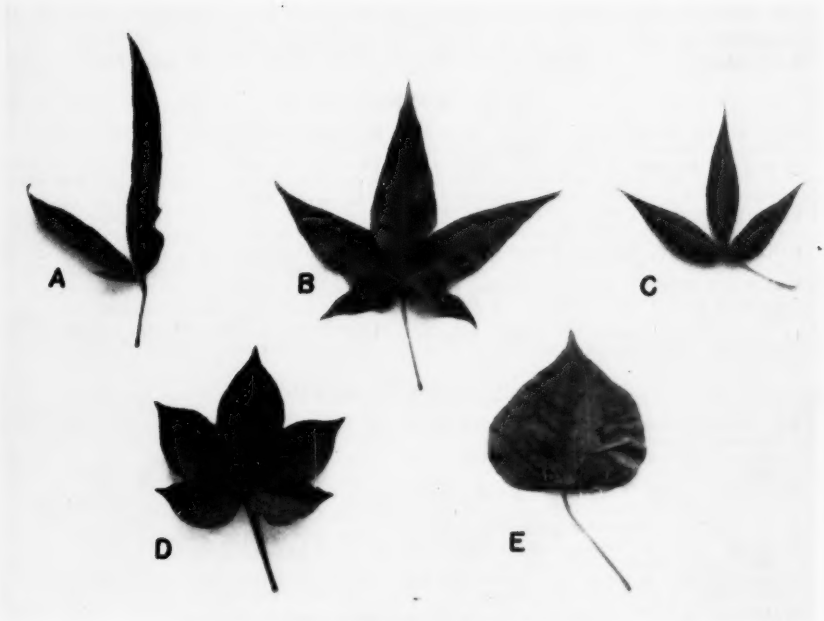

SEGREGATION IN COTTON LEAF SHAPES
(SEE PAGE 103)

Cytogenetics of Rubus
Five New Tomato Characters
Transplantation of Ova in Mice
Lethal Mutation in Dorking Fowl
Hydrocephalic Lethal in Herefords

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The

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Vol. L MAY-JUNE, 1959 No. 3

CONTENTS

INHERITANCE OF FIVE NEW TOMATO SEEDLING CHARACTERS
By Charles M. Rick and A. L. Harrison 91

RELATIONSHIP BETWEEN RECESSIVE OBESITY AND INDUCED PULMONARY TUMORS IN MICE
By George Vlahakis and W. E. Heston 99

SEGREGATION IN NEW ALLOPOLYPLOIDS OF *Gossypium*
By L. I. Phillips and D. U. Gerstel 103

TRANSPLANTATION OF OVA IN MICE
By Earl L. Green and Margaret C. Green 109

THE INHERITANCE OF LARVAL PIGMENTATION IN *Aedes aegypti*
By George B. Craig, Jr., and Nicholas W. Gillham 115

KENNETH WILLIAM NEATBY
By T. Johnson 125

HEREDITARY EXENCEPHALY IN THE FOWL
By W. J. Mellen 127

CYTOGENETICS OF *Rubus*
By D. Markarian and H. P. Olmo 131

A LETHAL MUTATION IN DORKING FOWL
By Walter Landauer 137

A NEW DOMINANT *T*-ALLELE IN THE HOUSE MOUSE
By Mary F. Lyon 140

A HYDROCEPHALIC LETHAL IN HEREFORD CATTLE
By R. L. Blackwell, J. H. Knox and Estel H. Cobb 143

Notes and Reviews

NATIONAL SCIENCE FOUNDATION RESEARCH PROPOSALS 108

POSITIONS OPEN FOR GENETICISTS 114

LEAVES AND PLANT OF THE MOUSE EARS MUTANT

Figure 1

A—Comparable leaves of normal and mutant plants. Normal on the left; *Me*/+ in the center; *Me*/*Me* at the right. *B*—Typical field-grown plant of *Me*/*Me*.

INHERITANCE OF FIVE NEW TOMATO SEEDLING CHARACTERS

CHARLES M. RICK AND A. L. HARRISON*

THE advantages for the study of tomato genetics that are offered by seedling character have been delineated previously[6]. From various spontaneous mutants acquired during the past few years, we have concentrated our efforts upon the group having well-defined seedling manifestation, of which five are reported here. The purpose of this article is to describe their morphology and to report the nature of their inheritance and linkage relations. New information concerning the loci of the previously described seedling characters *Cu*[7], *tf*[6], and *rv*[6] is also included.

The phenotypic deviations from normal are described in detail for certain of the mutants because they will probably prove useful for future research. The advantages common to this group are (1) marked juvenile manifestation, permitting unmistakable identification in seedling cultures, (2) normal viability, even when homozygous, and (3) sufficient fertility to permit inheritance studies. In addition, single and double doses of the reponsible gene can be detected for two of the group.

The five new mutants described herewith were discovered by us or were submitted to us by other workers. Details concerning the origin are given for each in the following section.

Descriptions of the Mutants

Me, Mouse ears (LA 324)

A single plant with highly modified leaves and peculiar dense growth was found in a field of var. Rutgers growing in Texas in the spring of 1953. Seed from this original plant produced seven normal plants and 11 with extreme leaf modifications similar to those of the parent. Some plants of the latter group yielded selfed progeny that bred true for the mutant character while others produced segregating progeny. Further experience revealed that two classes of the mutant phenotype could be readily distinguished in segregating families and that monogenic segregation was involved. The segregation in a representative family — 96 mutant: 175 intermediate: 89 normal — provides a remarkably good fit to the expected 1:2:1 ratio. Treating *Me* as a completely dominant mutant, the data of 11 families show a significant deficiency of *Me* and significant heterogeneity. But, after one family with a very great deficiency of *Me* is subtracted, the remaining 10 families do not deviate significantly from 3:1 (2060 *Me*: 746 +; $\chi^2 = 3.68$; P = 0.05-0.1) or from homogeneity ($\chi^2 = 16.54$; P = 0.05-0.1).

This gene manifests its effects primarily on leaf development. Leaves of the heterozygote are three to four times pinnately compound instead of the mostly once-pinnately compound condition of the normal leaf (Figure 1*A*). The number of first-order segments is increased. The ultimate segments are smaller, more distorted, curled, broader, and more remote from the rachis than normal. Some leaflets are compressed laterally, suggesting the effects produced by mild strains of cucumber virus. Leaves of the homozygote are still more modified in the aforementioned aspects except that they are less pinnatifid. Their shape is quite variable and subject to environmental influence. Foliage may become scanty from reduction of the ultimate segments, which often assume a broad, curly, deltoid shape, remarkably suggestive of the auricular appendage of mice, hence the name, Mouse ears, and symbol *Me*.

Internodes of both *Me*/*Me* and *Me*/+

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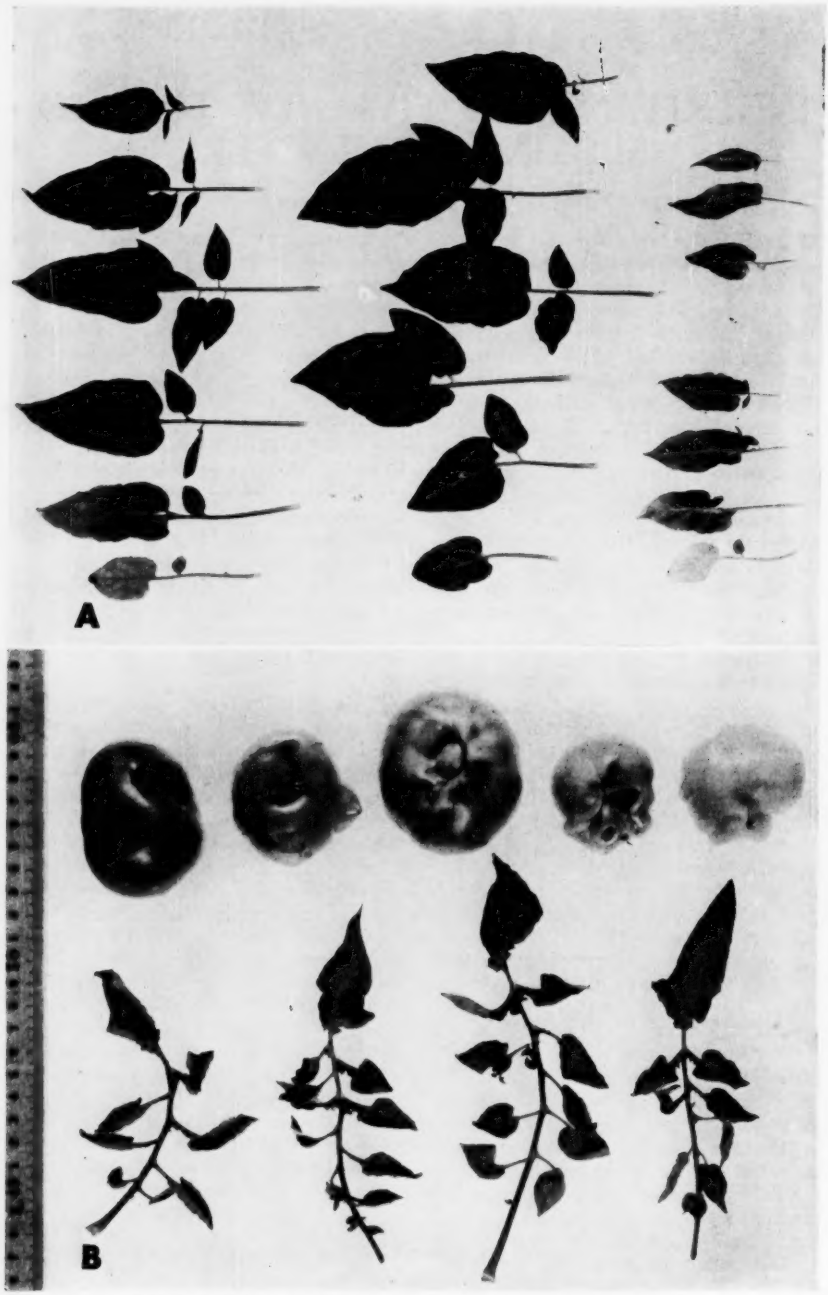

LEAVES AND FRUITS OF THE SOLANIFOLIA MUTANT

Figure 2

A—Array of seedling leaves of *sf* (left); *c* (center); and the *sf-c* compound (right). Each array is taken from a single plant, the lowermost being the first true leaf, progressing upward to the youngest leaf. *B*—Mature fruits (stylar end) and mature leaves of *sf*.

are somewhat shortened, accentuating the compact appearance imparted by leaf shape (Figure 1*B*). Gamete and seed fertility and natural fruiting are not seriously impaired by *Me*.

The seedling recognition of *Me* is remarkably good. With experience reasonably accurate classification of the three genotypes can be made in the primary leaves. It is therefore one of the few valuable dominant seedling mutants (*Wo*^{m}, *H*) that are classifiable into all genotypes and are both viable and fertile when homozygous.

sf, solanifolium (2-311)

This pleiotropic mutant was discovered as a single highly unfruitful plant in the variety Pearson growing in a commercial field near Woodland, California in 1955. Mutant plants comprised most of the small progeny grown from naturally produced seeds harvested from the original plant. As a seedling *sf* shows considerable resemblance to *c*, but differs in certain recognizable features. The first true leaf usually has large entire segments with a small basal lateral. This tendency toward a reduced number of leaf segments continues throughout seedling development but is never so extreme as in *c* (Figure 2*A*) or *e*. Margins of all leaves are entire; the leaf surface tends to be ribbed and to have chlorotic markings. The interactions with *c* and *e* in the seedling stage are complex but workable. Primary leaves of the compound *sf-c*, for example, are smaller than those of either mutant alone, having a single element and usually one or two tiny basal segments (Figure 2*A*).

The mature plant of *sf* has about the same habit and approaches the same size as normal plants of Pearson, but its peculiar leaves give it a distinct appearance. These organs have the normal number of larger segments, which, however, are darker green, entire, folded, and curled to reveal a considerable extent of the lower surface (Figure 2*B*). The smaller segments are usually lacking. The superficial resemblance to leaves of some of the tuberiferous species of *Solanum* suggested the name and symbol. The flowers are also strikingly modified (Figure 3*D*). Calyx and corolla segments are narrow and elongate. Stamens appear normal individually, but are irregularly disposed and frequently dialytic, so that the anther tube is seldom completely closed. Pistils are highly variable and usually defective, often being split into several parts, each carpel being surmounted by its own twisted style. Such defects persist in the fruit, none of which appears normal at maturity (Figure 2*C*). In view of such defects, it is remarkable that any flowers can naturally self-pollinate and set fruit, yet a small proportion do so and fertility in controlled crosses, particularly on the male side, is good.

Segregation data for six F_2 families from different crosses reveal a close fit to 3:1 (1688 + : 555 *sf*) with no significant departure from homogeneity ($\chi^2 = 9.78$; P = 0.05-0.1). The evidence for recessive monogenic inheritance is therefore good.

ah, Hoffman's anthocyaninless (LA 260)

A complete anthocyanin deficiency was discovered by Dr. I. C. Hoffman of the Ohio Agricultural Experiment Station, to whom we are indebted for this mutant. The material submitted to us consisted of lines of Globe A, Marhio, and Rutgers, each breeding true for this character. Sufficient information is not available to indicate whether the deficiencies arose separately in each line by mutation or they trace to a single original mutation, although the latter origin is more probable. Whatever the recent history, it is certain that the deficiency did not exist in the original parents of these lines. Appropriate testcrosses established that the genes in each line were allelic with each other but not with the other known anthocyanin deficiencies, *a*, *ag*, *al*, and *aw*. As far as we can ascertain under the various conditions that we have grown *ah*, it fails to develop purple pigment in any parts and is therefore phenotypically identical with *a* and *aw*.

Segregation in F_2 from eight different crosses was 2338 + : 704 anthocy-

FLOWERS, FRUITS, AND LEAVES OF VARIOUS MUTANTS

Figure 3

A—Mature fruits and leaves of *ht*. *B*—Mature fruits and leaves of a normal plant of the parent variety, San Marzano. *C*—Flowers of *ht*. *D*—Flowers of *st*. *E*—Flowers of the normal type. Scales all in cm.

LEAVES OF THE FILICIFOLIUM MUTANT
Figure 4

Typical leaves from F_2 segregating for the filicifolium (*ff*) gene. Normal leaf at left; *ff*/*ff* at right; *ff*+ in the center.

aninless, the moderate deficiency of mutants from a 25 percent expectation being statistically significant. One family of 452 seedlings contributes most of this discrepancy, for, when its values are subtracted from the total, the remainders —1960 + and 630 *ah*—provide a good fit to 3:1. Even with this family removed, however, the data show significant heterogeneity ($\chi^2 = 17.5$; $P < 0.01$), which is attributed to variable survival at germination. Its monogenic inheritance being established, this new mutant is named Hoffman's anthocyaninless after its discoverer and is assigned the symbol *ah*.

ht, hastate (2-295)

The stock of this mutant traces to an unfruitful plant of tremendous vigor encountered in a field of var. San Marzano near Clarksburg, California in 1955. A few seeds set naturally by this plant produced four normal plants and six of the original mutant type. Artificial selfing of the normal plants yielded a third generation of segregating progenies, and the mutant plants bred true. The total F_2 segregation thus far observed in 12 families is 2488 normal : 505 hastate. For a single recessive gene the deficiency of hastate is highly significant. The families are remarkably consistent in their deficiencies since a statistical test reveals no heterogeneity ($\chi^2 = 1.2$; $P > 0.99$). As this inheritance pattern is typical of weak recessives, it is likely that only a single gene is involved.

Hastate is immediately recognized in the first true leaf, which differs from normal in having an entire, elongate, strap shape. Among genes of similar effect—*c*, *e*, and *sf*—*ht* can be distinguished by its narrower and more elongate primary leaves. Its compounds with *c* and *e* have highly modified, reduced leaf blades. The distinction persists in leaves of the mature plant (Figure 3*A*), which are not nearly so condensed as in *c* and *e*, are smaller, and have fewer segments than normal. The leaf segments are more plicate and acuminate and, with

their usual two basal segments, are roughly halberd-shaped, suggesting the name and symbol for this mutant.

Most flower buds of *ht* abort before opening, and the few that open do so irregularly and incompletely (Figure 3*C*). Stamens are variable, often dialytic, twisted, discolored, or otherwise atypical. Pistils appear normal macroscopically, but fruits are more elongate, slender, and pointed than normal (Figure 3*A*). Pollen is usually present in small quantities, and *ht* can be used as staminate or pistillate parent in crosses without great difficulty.

ff, filicifolium (LA 509)

This fascinating mutant was discovered by Dr. Jens Roll-Hansen of Stjørdal, Norway and transmitted to us by Dr. Robert Lamm of Alnarp, Sweden. The effects of *ff* are manifest in leaf form, which is modified in a manner completely different from that of any other tomato mutant that we know of. In the seedling stage the first leaves of *ff* deviate from normal in being more highly divided with more acute segments and often having whitish-yellow marginal and interveinal areas. The tendency toward proliferation increases as subsequent leaves are developed, until a state of complex subdivision is reached in the mature leaf (Figure 4). Instead of the normal tomato leaf with second order segments, the *ff* leaf has well-developed third and even fourth order segments. A single lateral segment may present the same degree of complexity as the whole leaf of a normal plant. The ultimate segments, in contrast to those of *Me* and + terminate in very acute tips. Enations frequently appear on the *ff* corollas, but it is not known to what degree this character is associated with the modified leaf form. No other modifications have been observed, and the plant has normal fertility.

In heterozygotes similar, though much less extreme leaf changes can be observed (Figure 4). The ratio observed in our first F_2 (36 normal: 96 intermediate: 46 filicifolium) suggested monogenic inheritance and incomplete dominance. For most purposes, and particularly for seedling segregations, *ff* is best treated as a recessive. Five families from different crosses yielded the total segregation, 1393+ : 380 *ff*. The deficiency of *ff* is significant ($\chi^2 = 12.03$; 1 df) but the families are consistent in this deficiency (heterogeneity $\chi^2 = 4.15$; 4 df).

Linkage Relations

Each of the above described genes was subjected to linkage screening tests and four were successfully placed in their respective chromosomes. Stocks of each were crossed to multiple seedling marker stocks and F_1 and F_2 generations were grown. Data are reported here only for those progenies that gave a significant indication of linkage; for the records of other test segregations the reader is referred to reports by Rick[4].

Linkage values were determined mostly from repulsion F_2 progenies, using the product-moment method and deriving values from Immer's[3] useful tables. Because many of the monogenic ratios deviate significantly from expectation, this method is appropriate and suffices for the intended goal of finding the respective chromosomes.

Linkage tests, in addition to those of the newly described genes, are presented for three genes of chromosome 7 and for *Cu* since they clear up the previously existing uncertainties about their positions. The results will be presented in order of the number of each chromosome based on pachytene length[1].

Chromosome 2

Two of the genes reported here, in addition to *Cu*, fall in this chromosome. As Table I reveals, the large deviations from independent assortment indicate rather conclusively that these genes exist in chromosome 2.

Cu. This remarkable mutant reported by Young[7] behaved for us as a very useful dominant seedling marker, fully viable and fertile, but *Cu/Cu* being indistinguishable from *Cu/+*. The rather substantial deficiencies in the numbers of *Cu* segregants in certain families might be explained by the action of the hypothetical lethal *le*, which Butler[2] proposed to account for disturbed segregations on chromosome 2. According to the data in Table I, the locus of *Cu* lies very close to that of *Wo*. One crossover obtained in family 58L894 was of such phenotype as to suggest that *Cu* lies beyond *Wo* from d_1.

Me. More extensive data were obtained for this gene than for *Cu*. Although, as in the case of Wo^m, the three genotypes for this gene can be identified phenotypically, the present segregations were made in the early seedling stage, where such separations are less trustworthy than in the mature plant. By treating *Me* as a complete dominant throughout, however, no difficulty was encountered. The data

indicate no crossing-over between *Me* and *Wo*^{m}, but a few individuals were scored as *Me Wo*^{m}/*Me* +, in which the homozygous condition of *Me* and the heterozygosity of *Wo*^{m} seemed clearly defined. The relative distances between *Wo*^{m} and *Me* and the other markers suggest that *Me* lies on the same side of *Wo*^{m} as d_1. Family 58L261 gives rather consistent data in respect to the position of the four genes for which it is segregating: $d_1 - 12.5 - aw - 16 - Me - 3 - Wo^m$.

Considering the above linkage experience with *Cu*, it is quite a coincidence that *Me* also lies very close to *Wo*^{m}. These tests point up the need for an intensive study to ascertain more precisely the loci of all three genes.

ff. A significant indication of linkage was encountered in a single test between *ff* and *Wo*. Since random recombination was observed between *ff* and d_1, *ff* must lie beyond *Wo* from d_1 in a region which is not well marked at the present time. The sample is too small to permit much confidence in the estimated 35 units distance from *Wo*.

ht. Early tests suggested a linkage between *ht* and d_1, but these were not verified by subsequent F_2's, particularly those segregating for the markers, *m* and *Wo*, known to be located on either side of d_1.

Chromosome 7

A linkage between *tf* and a wilty gene was reported previously[6]. The wilty character was concluded to be different from the one conditioned by *wt* on chromosome 7 because an allele test between them yielded only normal plants. When recent trisomic tests[4] indicated conclusively that *tf* also lies on 7, the matter of wilty genes was re-examined. The allele cross was repeated and large field-grown F_1 and F_2 generations consisted entirely of *wt* plants, indicating that the two genes were allelic, if not identical. The first allele test progeny had been grown in the greenhouse, where conditions are sometimes unfavorable for the expression of this rather variable character.

With this new information concerning the position of *tf*, a three-point test was made between *wt*, *tf*, and *mc*, with the results summarized in Table I. The tests for detection of linkage are significant between *wt* and *tf*, and between *tf* and *mc*, but not between *wt* and *mc*. The calculated crossover distances agree in placing *tf* in the middle of this group as follows: $wt - 15 - tf - 30.5 - mc$. Chromosome 7 therefore has in *tf* a strategically placed and very well defined seedling marker.

TABLE I. Summary of linkage data

Pedigree number	Phase*	Chromosome	Gene combination		Observed frequencies				Contingency chi-square**	Estimated crossover value
			m	*t*	++	+ *t*	*m* +	*m t*		
58L894	R	2	*Cu*	d_1	10	21	59	19	16.1	26.5
	C		*Wo*^{m}	d_1	59	20	10	20	14.3	27.5
	R		*Cu*	*Wo*^{m}	1	30	78	0	99.3	0.5
57L572	R		*Cu*	d_1	41	31	160	54	7.1	39.0
57L688	R		*Cu*	d_1	71	67	311	101	57.1	35.5
57L219	R		*Me*	d_1	18	25	97	23	23.1	27.5
57L567	R		*Me*	d_1	36	32	134	28	20.6	31.0
57L686	R		*Me*	d_1	67	66	253	76	30.0	34.0
58L261	C		d_1	*aw*	200	33	18	113	178.5	12.5
	R		*aw*	*Me*	21	197	103	43	141.7	16.0
	R		d_1	*Me*	44	189	80	51	64.6	26.0
	R		*Me*	*Wo*^{m}	0	124	125	115	96.1	0
	C		*aw*	*Wo*^{m}	114	104	11	135	75.7	18.5
	C		d_1	*Wo*^{m}	105	128	20	111	31.7	29.5
58L262	C		d_1	*aw*	199	19	17	83	170.2	11.0
	R		*aw*	*Me*	20	196	88	14	179.8	10.0
	R		d_1	*Me*	38	180	70	30	82.2	21.5
58L1032	R		d_1	*Me*	44	156	70	24	71.9	22.0
	R		*Me*	*Wo*^{m}	0	114	66	114	51.8	0
	C		*O*	*Wo*^{m}	52	148	14	80	3.9	40.5
59L1327	R		*ff*	*Wo*	28	108	16	20	7.3	35.0
57L546	C	7	*wt*	*tf*	198	11	28	39	92.3	15.0
	R		*tf*	*mc*	226	60	47	3	8.7	30.5
	R		*wt*	*mc*	156	53	57	10	2.6	40.5
57L187	R	9	*ah*	*wd*	97	46	38	1	12.2	16.0
58L432	R		*ah*	*wd*	143	93	85	0	45.3	0
58L87	R	?	*sf*	*rv*	164	49	80	7	8.2	33.0
58L141	R		*sf*	*rv*	599	85	231	11	11.2	35.0

*C-Coupling; R-Repulsion

**All sets have 1 df; 5% level = 3.8; 1% level = 6.6

Chromosome 9

Two F_2 progenies segregating for *ah* and *wd* leave no doubt concerning the linkage between these two genes: in one family a single homozygous crossover individual was found; in the other, none. Among small F_3 progenies grown from F_2 *wd* segregants, 13 segregated for *ah*, 16 did not. The latter information provides an estimate of 29 units; the F_2's, 16 and 0. With such great discrepancies in estimates, a more critical determination of this linkage must await more intensive study. Having this linkage, we are now provided with a much more useful marker for chromosome 9 than *wd*, which requires special environmental conditions for its expression and cannot be readily classified in the seedling stage.

Undetermined chromosome

Many attempts have been made in the past to obtain a significant indication of linkage for *rv* before the present relationship was found with the new marker *sf*. The two estimates of map distance based on F_2 data in Table I are in remarkable agreement, being 33 and 35 units respectively. The chromosome on which these useful seedling markers are located has not yet been identified, but the genetic independence of both with markers on all other identified groups renders it likely that they lie on a separate chromosome.

Discussion

Efforts to find the chromosome corresponding to each of the four genes described in this article were successful. In addition, linkages are reported for three previously unlocated genes (*Cu, rv, tf*).

The placement of *ah* in chromosome 9 provides this chromosome with its first good seedling marker, and the linkage discovered for *sf* provides its group (probably an otherwise unmarked chromosome) with a better marker than *rv*. These discoveries, in addition to other linkages revealed recently by other workers in Reports of the Tomato Genetics Cooperative, bring closer to realization the goal of obtaining good seedling markers on each tomato chromosome. At present two chromosomes, possibly only one of the 12, lack such markers.

The number of chromosomes for which useful groups of linked seedling markers have been found is likewise steadily increasing. Thus, eight chromosomes are marked by at least two linked seedling genes apiece. Further linkage studies with these chromosomes should therefore be greatly expedited and the study of the effect of various agents upon crossing-over facilitated.

The present work also underscores a situation that had been previously sensed by Rick and Butler[4]; namely that a disproportionately large number of genes have been found for certain tomato chromosomes, particularly chromosome 2. Three of the seven genes tested herewith show linkage relations with other markers of this chromosome, bringing the total to 22 and including no less than 11 seedling markers. Various aspects of this tendency toward non-random distribution will be considered in another publication.

Summary

Phenotypes are described for five new mutants of the cultivated tomato. Each one can be clearly distinguished from normal in the seedling stage and is conditioned by a single gene. A complete anthocyanin deficiency results from the action of *ah*; leaf shape is modified in various ways by the other mutations. One (*Me*) is incompletely dominant, permitting identification of all three genotypes; another (*ff*) can be similarly classified under field conditions but in general is most effectively treated as a recessive; the rest (*sf, ah,* and *ht*) are recessive. Except for a consistent deficiency of *ht* and *ff* homozygotes and a few deviating families for other genes, the fits to expected ratios are good.

A significant indication of linkage was obtained for four of the genes and also for the previously unlocated genes, *Cu, rv,* and *tf*. The genes, *Cu, ff,* and *Me* exhibit linkage with markers of chromosome 2, for which a four-point testcross reveals the following spatial relations: d_1, — 12.5 — *aw* — 16 —*Me* — 3 — Wo^m. Chromosome 7 is marked by *tf*, a three-point test providing the following estimated distances: *wt* — 15 — *tf* — 30.5 — *mc*. A linkage between *ah* and *wd* provides chromosome 9 with its first known seedling marker. The gene *sf* is linked with *rv* (33-35 units) on an unidentified chromosome.

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RELATIONSHIP BETWEEN RECESSIVE OBESITY AND INDUCED PULMONARY TUMORS IN MICE

GEORGE VLAHAKIS AND W. E. HESTON*

OBESE (*ob*)[8], a recessive mutation which arose in the V stock of mice at the Roscoe B. Jackson Memorial Laboratory in 1949, offered the opportunity to study the effect of this gene on induced pulmonary tumors in the mouse. Since obese animals weigh considerably more than their non-obese litter mates, it seemed worthwhile to determine if any association exists between the gene obese (*ob*) and susceptibility to pulmonary tumors. In earlier reports[2,3,4] an association between susceptibility to induced pulmonary tumors in mice and the genes flexed-tail (*f*), shaker-2 (*sh-2*), waved-2 (*wa-2*), lethal yellow (A^y) and hairless (*hr*) was demonstrated, and there appeared to be a positive correlation between the relationship of these genes to occurrence of pulmonary tumors and their relationship to body weight. Lethal yellow increased body weight and increased susceptibility to both induced and spontaneous[5] pulmonary tumors, while hairless decreased body weight and was associated with a decrease in susceptibility to induced and spontaneous[6] pulmonary tumors. Later[7] it was shown that flexed-tail decreased body weight and decreased susceptibility to spontaneous pulmonary tumors, and shaker-2 was associated with a decrease in pulmonary tumors and with a decrease in body weight. The association between the waved-2 gene and susceptibility to pulmonary tumors was interpreted as possibly the result of linkage between waved-2 and shaker-2. Like shaker-2 and flexed-tail, waved-2 also reduced body weight, though not to the same extent. In respect to the A^y gene it has also been noted that the yellow mice are not only heavier than their non-yellow litter mates, but also exceed them in such measurements as total body length and tail length[1].

Data presented in this paper reveal an association between the gene obese, that greatly increases body weight because of the great amount of adipose tissue, and a reduction in number of pulmonary tumors, although a positive correlation was observed between pulmonary tumors and muscular and skeletal development as revealed by body length, length of femur, and weight of gastrocnemius muscle.

Materials and Methods

Strain A female mice genetically highly susceptible to pulmonary tumors were outcrossed to Vob males heterozygous for obese (*Obob*) to produce AVobF_1's. The Vob strain was obtained from the Roscoe B. Jackson Memorial Laboratory April 1, 1953. Since obese (*obob*) animals will not mate, the Vob strain is maintained by mating males and females that through breeding tests have been shown to be heterozygous for obese. Strain A has been used extensively in this laboratory in pulmonary tumor studies and is maintained by brother × sister matings, and now exceeds the 103rd inbred generation.

AVobF_1's, that through breeding tests had been shown to be heterozygous for obese, were mated to produce AVobF_2's. From a mating of F_1's heterozygous for obese, the following types of animals can be expected: ¼ homozygous non-obese (*ObOb*), ½ heterozygous non-obese (*Obob*), and ¼ homozygous obese (*obob*). At weaning (approximately one month of age), the F_2's were classified as to obese and non-obese, the obese animals being easily recognizable from their non-obese litter mates by their general

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obese appearance and particularly by their expansive hind quarters. The mice were kept in plastic cages 7″ × 11″ × 5″ high with eight animals to a cage, and segregated as to sex but not as to obesity. They were given an unlimited supply of tap water and NCI pellets*, and were weighed weekly.

From approximately two months of age the mice were given 12 weekly injections intraperitoneally of 12.5 mg. urethan dissolved in water. At four months after the first injection, the animals were killed (etherized) and examined for pulmonary tumors. Approximately 1 cc. of Fekete's modification of Tellyesniczky's fixative (70% alcohol, 20 parts; formalin, two parts; glacial acetic acid, one part) was injected into the lungs intratracheally before they were removed from the chest cavity. Lobes were separated and tumors appearing on the surface of each lobe were counted with the aid of a dissecting microscope. Immediately after etherization and before autopsy, measurement of total body length, from tip of nose to tip of tail, was taken, the left gastrocnemius muscle was dissected out and weighed, and the left femur was removed and the length measured with a pair of vernier calipers.

Results and Discussion

Nearly all the AVobF$_2$ mice had pulmonary tumors, but the differences were clearly shown by the number of tumors occurring in each animal. From an examination of Table I, it is seen that the non-obese mice had almost twice as many pulmonary tumors as did the obese mice. Non-obese males had a mean of 23.9 tumors and the obese males a mean of 12.3 tumors. The difference between the means of the two groups is statistically significant ($t = 3.778$; $P < .01$). The average weight of the non-obese males was 39.2 grams and that of the obese males was 64.3 grams. The difference of 25.1 grams between the two groups is highly significant ($t = 18.281$; $P < .01$). The difference between the non-obese and obese females in mean number of pulmonary tumors is also significant ($t = 2.301$; $.02 < P < .05$), and the difference of 39.7 grams between the average weights of non-obese and obese females is highly significant ($t = 18.878$; $P < .01$). When the males and females are combined the difference between non-obese and obese in respect to average number of tumors is highly significant ($t = 4.545$; $P < .01$).

Although the obese animals distinctly weighed more than their non-obese litter mates, they were actually smaller animals with respect to certain body measurements included in Table I. The average weight of the gastrocnemius muscle for the non-obese males was 0.1936 grams, and that for the obese males was 0.1565 grams. The difference of 0.0371 grams is statistically significant ($t = 6.072$; $P < .01$). A similar difference was observed in the females; the non-obese females had a heavier muscle ($t = 4.447$; $P < .01$). Femurs in non-obese males and females were on the average longer than the femurs in the obese animals (males, $t = 6.380$; $P < .01$; females, $t = 2.724$; $P < .01$). The difference in body length between non-obese and obese males is significant ($t = 3.766$; $P < .01$). The average body length of the non-obese females was slightly longer than that of the obese females, but the

TABLE I. AVobF$_2$ mice classified according to genotype or phenotype with averages for certain body measurements and for pulmonary tumors induced by 12 weekly intraperitoneal injections of 12.5 mg. urethan

Classification of mice	Number of mice	Mice with tumors	Average number of tumors	Average weight at time mice were killed	Average body length	Avg. wt. L. gastrocnemius muscle	Average length left femur
		percent		gms.	cm.	gms.	cm.
Ob— (non-obese) ♂♂	93	92	23.9	39.2	21.0	0.1936	1.63
obob (obese) ♂♂	46	85	12.3	64.3	20.4	0.1565	1.55
Ob— (non-obese) ♀♀	93	98	29.3	30.4	20.3	0.1588	1.60
obob (obese) ♀♀	31	94	19.5	70.1	20.2	0.1406	1.53

*The National Cancer Institute pellets (formerly referred to as Derwood pellets) are prepared by Herbert Bryant, Inc., Gaithersburg, Maryland, and are composed of the following: skim-milk powder (human grade), 23.29%; ground whole hard wheat, 65.1%; dried debittered brewer's yeast, 4%; corn oil, 5.8%; sodium chloride (iodinated), 1.4%; iron citrate, U.S.P. #7 powder, 0.13%; shark-liver oil (4.8 million units Vitamin A per pound), 0.2%; and Delstrol® (900,000 units Vitamin D per pound), 0.08%.

difference is not statistically significant ($t = 0.528$; $.5<P<.6$).

Pearson product-moment coefficients of correlation were computed and are shown in Tables II and III. To ascertain whether the observed correlations differed significantly from zero, t tests were employed. The t values are listed along with the associated probabilities in the Tables. Correlations followed by a double asterisk (**) were considered significant, i.e., when the probability of a correlation differing from zero was more than 99 percent. Correlations followed by a single asterisk (*) were considered probable, i.e., when the probability of a correlation differing from zero was less than 99 percent but greater than 95 percent. The figures in parentheses refer to the total number of animals in each case.

Coefficients of correlation were determined between number of pulmonary tumors and total body length, length of femur, and weight of gastrocnemius muscle first for all the males and for all the females (Table II). It can be noted that in each case the correlation is positive and in each case it is highly significant. Thus, an increase in number of tumors occurred with an increase in normal growth expressed in these three measurements. Supporting this over-all correlation is the fact that the obese mice had significantly fewer tumors than the non-obese mice and were significantly smaller in respect to these three body measurements.

It was next desirable to ascertain whether or not these positive correlations prevailed when the data were broken down into the obese and non-obese groups (Table III). It was found that even after the effect of the gene obese had been eliminated a positive correlation existed between number of tumors and the two skeletal measurements and the muscle weight in all groups, and although the total number of animals in each of these groups was small, the correlation was highly significant in some groups.

It is also interesting to note the correlation between number of tumors and body weight when the variation in body weight due to the obese gene is eliminated (Table III). A positive correlation was found in both the non-obese and the obese groups of males and in the latter group it was statistically significant.

TABLE II. Coefficients of correlation between pulmonary tumors and various body measurements in combined non-obese and obese AVobF$_2$ mice. Figures in parentheses refer to the total number of mice

Group	Pulmonary tumors — total body length	Pulmonary tumors — weight of gastrocnemius muscle	Pulmonary tumors — length of femur
Ob— & *obob*	$r = +.24^{**}$	$r = +.33^{**}$	$r = +.30^{**}$
Males	$t = 2.893$	$t = 4.091$	$t = 3.667$
(139)	$.001 < P < .005$	$P < .001$	$P < .001$
Ob— & *obob*	$r = +.25^{**}$	$r = +.28^{**}$	$r = +.24^{**}$
Females	$t = 2.791$	$t = 3.221$	$t = 2.730$
(124)	$.005 < P. < .01$	$.001 < P < .005$	$.005 < P. < .01$

TABLE III. Coefficients of correlation between pulmonary tumors and the various body measurements in AVobF$_2$ mice segregated as to non-obese and obese. Figures in parentheses refer to the total number of mice

Group	Pulmonary tumors — body weight	Pulmonary tumors — total body length	Pulmonary tumors — weight of gastrocnemius muscle	Pulmonary tumors — length of femur
Ob—	$r = +.13$	$r = +.17$	$r = +.18$	$r = +.22^{*}$
Males	$t = 1.231$	$t = 1.606$	$t = 1.745$	$t = 2.151$
(93)	$.20<P<.25$	$.10<P<.20$	$.05<P<.10$	$.025<P<.05$
obob	$r = +.35^{*}$	$r = +.48^{**}$	$r = +.45^{**}$	$r = +.15$
Males	$t = 2.478$	$t = 3.629$	$t = 3.343$	$t = 1.006$
(46)	$.01<P<.02$	$P<.001$	$.001<P<.005$	$.30<P<.40$
Ob—	$r = +.034$	$r = +.24^{*}$	$r = +.085$	$r = +.18$
Females	$t = 0.325$	$t = 2.358$	$t = 0.814$	$t = 1.745$
(93)	$.70<P<.80$	$.02<P<.025$	$.40<P<.50$	$.05<P<.10$
obob	$r = -.34$	$r = +.34$	$r = +.43^{*}$	$r = +.089$
Females	$t = 1.947$	$t = 1.947$	$t = 2.564$	$t = 0.481$
(31)	$.05<P<.10$	$.05<P<.10$	$.01<P<.02$	$.60 <P<.70$

This suggests that without obese, factors causing a positive correlation with the other three body measurements are also effective in respect to body weight in males. Among the females, however, the correlation between number of tumors and body weight was positive in the non-obese group and negative in the obese group although not significant in either group.

It thus appears that in general, susceptibility to pulmonary tumors may be correlated with normal growth of the skeletal and muscular system instead of body weight when the weight is greatly influenced by adipose tissue. In addition to the correlations in the present study, it should be pointed out that the lethal yellow gene which has formerly been shown to increase the number of pulmonary tumors not only increases total body weight but also increases skeletal growth.

In somatotype studies Sheldon and coworkers[9] have found that women most likely to develop breast cancer are those with good skeletal and muscular development with some tendency toward obesity rather than the very obese women who are low on the mesomorphic scale. It would be interesting to know what correlations exist in man in respect to other neoplasms, particularly lung cancer, and somatotypes. In this laboratory, studies on these genes which have a marked effect upon body measurements in the mouse are being extended to tumors other than pulmonary tumors.

Summary

In a group of AVobF_2 mice segregated in respect to recessive obesity, and injected with urethan, *Ob*—(non-obese) males and females had more pulmonary tumors than did the *obob* (obese) animals. The obese males and females weighed much more than the non-obese males and females because of the excessive amount of adipose tissue in the obese animals, but the non-obese males and females exceeded the obese males and females in the total body length, length of femur, and weight of gastrocnemius muscle. The differences were highly significant except for the total body length of the females.

There were positive correlations between number of pulmonary tumors and body length, weight of gastrocnemius muscle, and length of femur for all groups, and they were highly significant in the combined obese and non-obese groups.

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SEGREGATION IN NEW ALLOPOLYPLOIDS OF GOSSYPIUM

III. Leaf Shape Segregation in Hexaploid Hybrids of New World Cottons

L. L. PHILLIPS AND D. U. GERSTEL*

STUDIES of artificially produced amphiploids were begun in this laboratory several years ago to further the understanding of their meiosis and genetic segregation. The first published account[1] of this work was concerned with hexaploid hybrids between *Gossypium hirsutum*, a cultivated cotton species of the New World, and two of its wild American relatives, *G. raimondii* and *G. thurberi*. Only segregation at the R_1 locus was then described. This locus is carried by tetraploid *G. hirsutum* (formula AADD) in the D genome; since *G. raimondii* and *G. thurberi* are diploid species possessing D genomes (DD) the artificially synthesized hexaploids (AADDDD) had four D genomes and carried two R_1 alleles from *G. hirsutum* and two from one of the wild species. In crosses made with recessive testers these hexaploids were found to segregate; consequently, they were not allopolyploids in a strict sense. However, the observed segregation ratios were far wider than the tetrasomic ratios characteristic of autopolyploids. The results from *G. hirsutum* × *raimondii* and *G. hirsutum* × *thurberi* hexaploids differed conspicuously insofar as those with *G. thurberi* gave even wider ratios than those synthesized with *G. raimondii*. It was suggested that these segregation differences were due to dissimilar degrees of preferential pairing between the chromosomes of *G. hirstum* and those of the two wild American species.

But before drawing any definite conclusions, it was necessary to consider to what extent the observed genetic ratios were influenced by irregularities in chromosome disjunction or by selective elimination of gametes and zygotes. Since sterility was high, irregularities might be suspected. One group of families was suitable for a genetic study of the regularity of disjunction at meiosis; it was found that the vast majority of functional gametes produced by a hexaploid parent had resulted from regular two-by-two disjunction of the chromosome set† carrying alleles at the R_1 locus. Out of a total of 556 gametes only seven carried but one chromosome of the set, two had three and the remaining 547 had the normal two chromosomes (table 5[1]).

The effect of selective elimination could be studied in two ways. First, it was found that the results of reciprocal crosses did not differ significantly and thus did not indicate any pronounced gametic elimination. Secondly, the survival of offspring produced from the two homogenic types of gametes was compared; plants resulting from R_1R_1 gametes functioned more often than those from $R^{RA1}R^{RA1}$ which indicated that some selection must have occurred. This was more likely due to zygotic selection, since the previous test for gametic selection had given a negative result.

Some of the families used in the earlier investigation and a few additional ones were available for a study of segregation at the leaf shape locus *L*. This locus is carried in the New World cottons on a D chromosome like R_1 and is not linked with the latter[6]. It is now of

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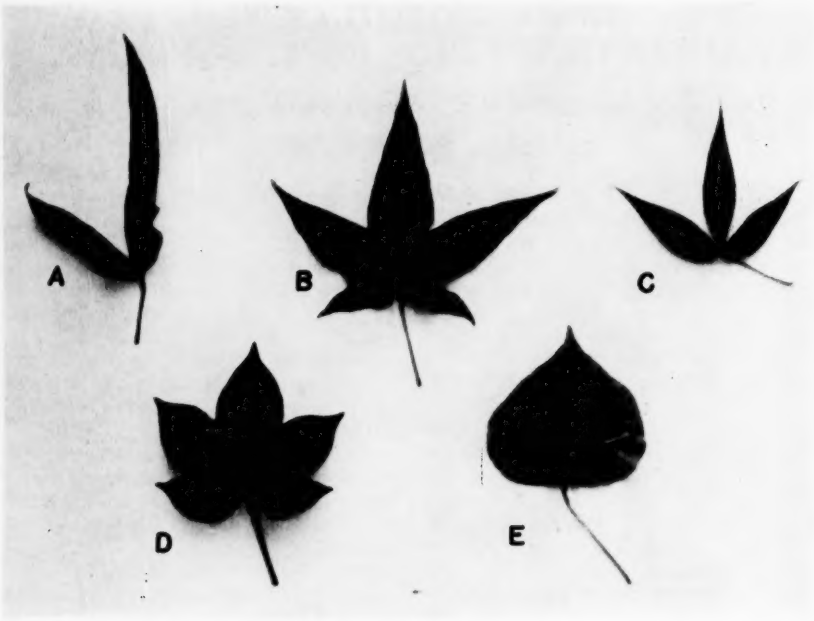
†"Set" is used here to designate the homologous or partially homologous chromosomes which can replace each other in the meiosis of a polyploid; see the preceding paper in this series[2].



PARENTAL LEAF SHAPES

Figure 5

A—L^s (G. hirsutum); B—L^o (G. hirsutum); C—l^{THU} (G. thurberi); D—l (G. hirsutum); E—l^{RAI} (G. raimondii).

interest to compare the behavior of different sets of chromosomes and therefore the present paper is devoted to a comparison of leaf shape segregation frequencies with those reported earlier for anthocyanin coloration.

Genetic Materials

Below are listed the various leaf shape alleles and the strains carrying them, starting with the narrowest and most deeply lobed type. Most of the symbols used for the cultigens are those of Knight's recent compilation[4]. The symbols l^{THU} and l^{RAI} are employed here for the first time; only for the first one has the existence of an active allele been shown[3].

The "superokra" allele L^s which originated in *G. barbadense* and is now also available in closely related *G. hirsutum* produces a degree of leaf lobing so extreme that leaves are either compound or reduced to a single narrow leaflet corresponding to the middle lobe of a typical leaf (Figure 5*A*). This allele is partially dominant in intra- as well as interspecific crosses.

The "okra" allele L^o produces a deeply palmately lobed leaf (Figure 5*B*). This allele is also partially dominant. It is carried in the *G. hirsutum* marker strain S 4025 which was used in allopolyploids with *G. raimondii* and *G. thurberi* of the previous study[1]. The L^o segregations in this paper were collected from the same progenies which had been scored for R_1 alleles by Gerstel[1]. The studies of the L^s allele reported below were made on hexaploids synthesized

from different *G. hirsutum* (and *G. barbadense*) stocks since L^s was not available in S 4025.

The *thurberi* allele l^{THU} produces in *G. thurberi* lobing of about the same depth as L^O in *hirsutum* (Figure 5*C*); on a *hirsutum* background its expression is weaker and much shallower sinuses are produced by this gene even in homozygotes[3].

The *hirsutum* tester lines contained recessive broad (*l*; Figure 5*D*) which causes relatively weak lobing. Commercial Upland cottons and the marker strain S5082 were employed.

G. raimondii possesses an entire leaf (l^{RAI}; Figure 5*E*); in *G. hirsutum* × *raimondii* the leaf shapes of the tetraploid species are almost completely dominant.

The synthesis of hexaploids and methods used in obtaining their offspring were described in the previous publication. As is usual for new allopolyploids, fertility was far from complete and on the average only one-fourth of the ovules matured. Pollen sterility also was found to be high; accurate determinations could not be made because of great fluctuations even between flowers taken from the same plant on the same day. *G. hirsutum* × *raimondii* hexaploids were more male sterile but had a better seed set than 6*x* (*G. hirsutum* × *thurberi*)[7].

Since the loci discussed are carried by *G. hirsutum* in the D-genome the artificial hexaploids of the constitution AADDDD are tetrasomic for chromosomes carrying these loci. For this reason they are characterized by tetrasomic formulae (e.g. $L^OL^O|RAI|RAI$); trisomic formulae (e.g. L^OL^Ol) are used for the pentaploids resulting from crosses AADDDD × AADD. No segregating genes are located in the chromosomes of the A genomes.

TABLE I. Leaf shape segregation in 6*x* (*G. hirsutum* × *raimondii*) × *hirsutum*

Plant No.	Narrow	Intermediate	Broad
Female segregation: $L^OL^O\|RAI\|RAI \times ll$			
S5094-1	7	174	14
S5094-2	21	216	19
5 plants*	8	126	17
Male segregation: $ll \times L^OL^O\|RAI\|RAI$			
3-113-B	15	166	13
Total	51	682	63

*Data from five small families combined.

Results

1. 6*x* (*G. hirsutum* × *raimondii*)

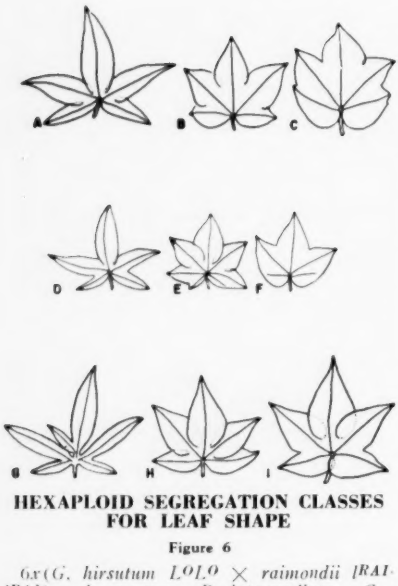
Seven artificially synthesized hexaploid individuals 6*x*(*G. hirsutum* L^O × *raimondii*) of the constitution $L^OL^O|RAI|RAI$ were available for crosses with a broadleaved (*ll*) tester strain of *G. hirsutum*. Three distinct types could be recognized among the offspring with the following frequencies (totals): 36 narrow : 516 intermediate : 50 broad leaved plants (Table I). Typical climax leaves[5] of the three types are pictured in Figure 6. Plants of the three types were again crossed to broadleaved (*ll*) *G. hirsutum* in order to ascertain their genetic differences. Plants of the pentaploid generation were used as males in these testcrosses in order to minimize the confounding effects that transmission of extra chromosomes might be expected to cause. Because of the wide range of phenotypic expression in the progeny of pentaploid interspecific hybrids visual scoring was not relied on but rather the ratio of leaf sinus depth to lobe length was measured[5]. The results are listed in Table II. Two distinct

TABLE II. Progeny test of the three pentaploid *G. hirsutum* × *raimondii* phenotypes (*ll* × 5*n* ♂)

Sinus/lobe ratios*	Frequencies of offspring from: Narrow ♂ parents		Intermediate ♂ parents		Broad ♂ parents	
.5 —.10	1		—		—	
—.15	1		3		—	
—.20	5		23		—	
—.25	13		26		—	
—.30	7	39	17	73	—	
—.31	2		2		—	
—.32	3		1		—	
—.33	2		1		—	
—.34	2		—		—	
—.35	3		—		—	
† —.36	—		—		—	
—.37	—		—		—	
—.38	—		—		—	
—.39	—		3		—	
—.40	—		3		—	
—.45	2		30		3	
—.50	9	31	32	94	4	23
—.55	12		17		7	
—.60	6		5		8	
—.65	2		4		1	

*Broadness increases from top to bottom of this scale.

†Intervals are smaller between .30 and .40 to indicate more clearly the break between the two progeny classes.



HEXAPLOID SEGREGATION CLASSES FOR LEAF SHAPE

Figure 6

6x(G. hirsutum L^OL^O × raimondii [RAI/RAI]). A—narrow; *B*—intermediate; *C*—broad. *6x(G. hirsutum L^OL^O × thurberi [THU/THU]). D*—narrow; *E*—intermediate; *F*—broad. *6x(G. hirsutum L^SL^S × thurberi [THU/THU]). G*—narrow; *H*—intermediate; *I*—broad.

ranges were observed with a gap from .36 to .38. This gap corresponded to the mean value of .37 which was characteristic of pentaploids of the intermediate L^O[RAI/l] range from .32 to .41 for individual leaves. In other words, the test progenies consisted mainly of broad (*ll* and indistinguishable from it [RAI/l]) and narrow (L^Ol). The unusually wide range of expression, not found in papers dealing with leaf shape segregation in pure species (e.g.[5]) must be ascribed in part to the simultaneous segregation of modifier systems from the two species as well as to the occasional transmission of extra chromosomes.

The broad pentaploids gave only broad offspring (last column) and were therefore of the constitution [RAI/RAI/l]. The results of the progeny tests of the narrow and intermediate parents were less convincing. The former gave more narrow than broad progeny and the latter more broad than narrow, as expected, but the difference between the two ratios of 39 narrow : 31 broad and 73 narrow : 94 broad was not significant ($.1>P>.05$). Yet, the narrow and intermediate leaf shapes of the pentaploid parents were clearly distinct (Figure 6) and undoubtedly of the genotypes L^OL^Ol and L^O[RAI/l], respectively. The explanation for the indecisive progeny test must lie in preferential pairing of *G. hirsutum* chromosomes. Such preferential pairing between the two chromosomes of *G. hirsutum* origin in the intermediate L^O[RAI/l] would tend to shift the trisomic ratio of 1 narrow : 2 broad in the direction of a 1:1 ratio, with L^O going to one pole and *l* to the other in most meiotic anaphases. In the narrow L^OL^Ol pentaploid preferential association is not expected if all three homologues are unchanged and of *G. hirsutum* origin. But this is unlikely, since L^OL^O gametes must have been formed by the hexaploid parent consequent upon nonpreferential L^O[RAI] association and crossing-over. Thus, if one L^O bearing chromosome of the pentaploid had obtained a *G. raimondii* segment by crossing-over in the preceding generation some preferential pairing might feasibly occur between the other L^O and the *l* bearing chromosomes; with nontransmission of trisomes the ratio would then be shifted from 2 narrow : 1 broad in the direction of a 1:1 ratio also. More work is desirable to clear this up; especially, a large number of narrow pentaploids should be tested to see whether they contain L^O bearing chromosomes of different composition and give distinct progeny ratios. Trisomic transmission, while it could explain the offspring obtained from intermediate pentaploids is apparently no major factor as it would shift the progeny ratio of the narrows from 2 narrow : 1 broad towards 5 : 1.

2. *6x (G. hirsutum × thurberi)* and *6x (G. barbadense × thurberi)*

Hexaploid hybrids combining *G. thurberi* with *G. hirsutum* L^O (five hexaploids), *G. hir-*

TABLE III. Leaf shape segregation in *6x (G. hirsutum × thurberi)* and *6x (G. barbadense × thurberi)*

Hexaploid	No. families†	Narrow	Intermediate	Broad
G. hirsutum L^O × thurberi	5	19	316	7*
G. hirsutum L^S × thurberi	3	27	468	21
G. barbadense L^S × thurberi	2	49	823	34
Totals (lines 2 and 3)		76	1291	55

*The seven plants in this class had leaves as broad as those of the tester parent. They probably did not receive leaf shape alleles from their hexaploid parent due either to chromosome elimination or to anomalous disjunction following intergenomic (AD) pairing (see text).

†Progeny from different hexaploids (= families) of each type were combined since they gave similar results.

sutum L^S (three hexaploids), and the closely related *G. barbadense* L^S (two hexaploids) were available for segregation analysis. The *G. hirsutum* L^O × *thurberi* hexaploids were crossed as females to a broad leaf *G. hirsutum* strain. The other two hexaploid types would not produce bolls when crossed to tetraploid testers so they were testcrossed with one of several different broad leaf hexaploids.

G. hirsutum L^O × *thurberi* hexaploids gave a leaf shape segregation of 19 narrow : 316 intermediate : 7 broad (Table III). Further analysis of leaf shape measurements (lobe length and sinus depth) led us to doubt the validity of the three classes, inasmuch as the individuals scored as "broad" (thought to be $l^{THU}l^{THU}$) had leaves as broad as their *G. hirsutum* (ll) parent (compare Figure 5*D* with Figure 6*F*). This result was unexpected because the l^{THU} allele, even in single dose in $l^{THU}l$ heterozygotes of *G. hirsutum*, results in a narrower leaf shape than ll[3]. We thus entertained the possibility that the plants scored as "broad" were actually the result of aberrant gametes from their hexaploid parent, and further, that the true $l^{THU}l^{THU}l$ plants were indistinguishable from "intermediate" ($L^Ol^{THU}l$) and thus were scored as "intermediates." The genotypes of the "broad" plants were more likely - $l^{THU}l$ or - - l, having received one or none of the alleles from the hexaploids. These individuals could have resulted from chromosome elimination; another possibility is that irregularities resulting from intergenomic (A-D) pairing could have occurred. Further study of this problem is now in progress.

Since the difficulty mentioned above was due in large part to the similarity in the expressions of the L^O and l^{THU} alleles, another allele (L^S) giving the leaf an extreme degree of lobing much more pronounced than that resulting from l^{THU} was introduced into *G. thurberi* hexaploids. The progenies from several such 6*x* plants of the type $L^SL^Sl^{THU}l^{THU}$ were easily classified into 76 narrow : 1291 intermediate : 55 broad (Table III), these classes corresponding to plants having L^SL^S, $L^Sl^{THU}l$ and $l^{THU}l^{THU}l$ alleles, respectively (Figure 6 *G*, *H* and *I*). It remains a moot question whether the broad class here contained any exceptional types similar to those discussed above.

Discussion

The pertinent results of the previous paper on the R_1 anthocyanin locus[1] are compared in Table IV with the present data on leaf shape segregation. Combined male and female segregation ratios were very much alike in *G. hirsutum* × *raimondii* hexaploids for the two loci, namely 1 : 11.9 : 1 and 1 : 12.0 : 1 for R_1 and L^O respectively. In the calculation of these ratios the frequencies of the two homogenic types of gametes contributed by each parent were considered equal. However, as mentioned in the previous paper, R_1R_1 and $R^{RAI}R^{RAI}$ eggs, or more likely the zygotes formed by them, did not survive with equal frequency. No significant difference could be shown between L^OL^O and $l^{RAI}l^{RAI}$, though there was some inequality between the classes. It is peculiar that in the case of the anthocyanin allele plants carrying two *G. raimondii* alleles were deficient, while in the leaf shape segregation $l^{RAI}l^{RAI}$ was represented more often.

As in the previous report, the segregation ratios in the hexaploids with *G. thurberi* were far wider than in 6*x*(*G. hirsutum* × *raimondii*). In the latter hexaploid the two loci have similar segregations; in the *G. thurberi* combinations there was a highly significant difference between segregations for the two loci. Here the R_1 locus gave a ratio 1 : 47.0 : 1, whereas leaf shape segregation amounted to 1 : 19.7 : 1. Heretofore, different loci in a particular polyploid had given similar ratios (see, e.g.,[2]). The significance of this novel

TABLE IV. Comparison of segregations for anthocyanin* and leaf shape in *G. hirsutum* × Wild American hexaploids

					Ratio†	Probability of equality of homogenic classes
6*x*(*G. hirsutum* × *raimondii*)						
Female segregation:						
54 R_1R_1	:	463 R_1R^{RAI}	:	30 $R^{RAI}R^{RAI}$	1 : 11.0 : 1	Less than .01
36 L^OL^O	:	516 L^Ol^{RAI}	:	50 $l^{RAI}l^{RAI}$	1 : 12.0 : 1	.2 — .1
Male segregation:						
15 L^OL^O	:	166 L^Ol^{RAI}	:	13 $l^{RAI}l^{RAI}$	1 : 11.8 : 1	.99 — .95
		Heterogeneity† $\chi^2 = .274$; df = 4; $P > .99$				
6*x*(*G. hirsutum* × *thurberi* and 6*x*(*G. barbadense* × *thurberi*)						
Female segregation:						
9 R_1R_1	:	588 $R_1{}_rTHU$	:	16 ${}_rTHU{}_rTHU$	1 : 47.0 : 1	.2 — .1
76 L^SL^S	:	1291 L^Sl^{THU}	:	55 $l^{THU}l^{THU}$	1 : 19.7 : 1	.1 — .05
		Heterogeneity† $\chi^2 = 15.96$; df = 2; $P < .001$				

*The data for anthocyanin segregation were taken from Gerstel[1]. Nine exceptional plants listed there are omitted here.

†Calculated under the assumption of equality of the classes derived from homogenic gametes.

observation will be the subject of further study. It may be noted that the narrower ratio for the *L* locus, as compared with the wider one for R_1, was obtained not only from the 6*x*(*L*^{8}-*thurberi*) stocks (Rows 2 and 3, Table III), but also from 6*x* (*L*^{0}-*thurberi*) which segregated for both the *L* and R_1 loci (Row 1, Table III and Row 4, Table IV).

That selective elimination of gametes or zygotes is not important in the hexaploids with *G. thurberi* is shown by the chi-square values in Table IV, indicating that the homogenic gametes are recovered in both the R_1 and the *L* segregations with about equal frequency. The frequency of offspring from exceptional gametes with one or three, instead of two, *L* alleles could not be studied since unfortunately only three progeny classes were distinguishable and not seven as for the R_1 alleles.

Summary

Hexaploid hybrids of New World cottons with *G. raimondii* and with *G. thurberi* produced widely differing segregation ratios for leaf shape. Testcross ratios were 1 : 11.9 : 1 in one case and 1 : 19.7 : 1 in the other. In agreement with an earlier report on anthocyanin segregation 6*x*(*G. hirsutum* × *raimondii*) gave the narrower and *G. thurberi* combinations gave the wider ratio. The observations suggest the occurrence of some differential chromosome affinity in the combination with *G. raimondii* and a higher degree of preferential pairing in hexaploids between New World cottons and *G. thurberi*.

The results differed from those previously obtained in that two different loci in the same hexaploid gave diverse gametic ratios. New World × *G. thurberi* hexaploids produced a 1 : 47.0 : 1 ratio for anthocyanin and 1 : 19.7 : 1 for leaf shape characteristics.

The above data were obtained by visual scoring of clearly distinctive phenotypes. Progeny testing was attempted in the case of the offspring from 6*x*(*G. hirsutum* × *raimondii*); full corroboration could not be secured in this way because the ratios among the later offspring were again complicated by differential pairing.

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The Division of Biological and Medical Sciences of the National Science Foundation announces that the next closing date for receipt of basic research proposals in the Life Sciences is January 15, 1960. Proposals received prior to that date will be reviewed at the spring meetings of the Foundation's advisory panels and disposition will be made approximately four months following the closing date. Proposals received after the January 15, 1960 closing date will be reviewed following the summer closing date of May 15, 1960.

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TRANSPLANTATION OF OVA IN MICE

An Attempt to Modify the Number of Presacral Vertebrae

EARL L. GREEN AND MARGARET C. GREEN*

IN mice, the number of vertebrae between the cranium and the sacrum may be 25, 26, or 27. Even within inbred strains there is some variability, but each strain of mice has characteristic percentages of numbers of presacral vertebrae. No strain is known to have only one number[3]. Hybrids between strains also have characteristic percentages of numbers of presacral vertebrae which are usually intermediate between those of the parental strains[2,3].

The two classes of hybrids produced by reciprocal crosses between inbred strains of mice should be genetically identical except for sex-linked genes in males. Despite the genetic similarity, offspring of reciprocal crosses sometimes are different in number of presacral vertebrae[5]. Since the difference occurs in both sexes, it cannot be due exclusively to sex-linked genes. It must be due to a "maternal effect," exerted either through the cytoplasm of the ova or through the uterine environments provided by the mothers. It is possible to discriminate between these two alternatives by transplanting the two kinds of ova into uteri of a third strain of mice. If the hybrids produced in the common uterine environment of the prenatal foster mothers are different, the cytoplasms of the ova are probably the cause of the "maternal effect." If not different, the uterine environments are probably the cause.

An experiment to discriminate between these two alternatives has been performed. A preliminary analysis[4,7] of the data indicated that the maternal effect was due to the uterine environments. A more complete analysis, with the sexes separate, has revealed that the difference between the two kinds of hybrids is larger in females than in males and that the difference between the hybrids is due to differences in the cytoplasm of the ova of the parental strains. The experiment, however, was not large enough to be definitive.

Methods

The two strains crossed reciprocally were DBA/1JGn (called DBA) and C57BL/10JGn (called C57). These strains were chosen (1) because a preliminary experiment had shown that the hybrids (DBA ♀/C57 ♂ and C57 ♀/DBA ♂) had different frequency distributions of the number of presacral vertebrae and (2) because the coat color genes in these strains would identify the offspring produced from transplanted ova. The hybrids between these two strains were "patroclinous," whereas the hybrids of C3H and C57BL, earlier reported[5], were "matroclinous."

Two experiments were carried out. In one, mice of the two strains were crossed reciprocally and the vertebral types of the offspring were recorded. These are the *nontransplanted* hybrids. In the other, ova of each of the two strains were fertilized by sperm of the other strain, after the ova had been transplanted into females of the BALB/cJGn (called BALB) strain. These are the *transplanted* hybrids.

The number of presacral vertebrae (psv) in mice of these two strains may be 25 or 26 or asymmetrical intergrades of 25 and 26, in which the 26th vertebra looks like a lumbar vertebra on the right (or left) and like a sacral vertebra on the left (or right) side. Each mouse was classified as one of these four types. For

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convenience in analyzing the data, the four classes were (with exceptions noted later) reduced to two. The mice were classified as having 25 presacral vertebrae (25 psv) or as not having 25 presacral vertebrae (not-25 psv), the latter class containing about equal numbers of mice with 26 presacral vertebrae and with asymmetrical combinations of 25 and 26. This classification results in some loss of information, but produces a binomial variable which is statistically more tractable than a trinomial variable (25, asymmetrical, 26) or than two binomial variables (25 *v.* 26 on right, 25 *v.* 26 on left)[3]. The method of preparing skeletons for study has been described by Green[6].

The mice produced from the transplanted ova must be distinguishable from the mice produced from the native ova of the recipient females. This requirement was fulfilled by using females of the BALB strain as recipients. The pertinent coat color genes in the ova of the BALB females are *Ab* (agouti, brown). If BALB ova are fertilized by C57 sperm, carrying *aB* (nonagouti, black,) the offspring are *AaBb* (agouti, black) and are rejected. If BALB ova are fertilized by DBA sperm, carrying *ab* (nonagouti, brown) the offspring are *Aabb* (agouti, brown) and are also rejected. Only those offspring produced by C57 ova and DBA sperm or the reverse will be *aaBb* (nonagouti, black). The differences in color are detectable within a few days after birth.

The procedure was to collect unfertilized ova from a female of one of the parental strains (C57 or DBA) and to transplant them into a BALB female which had just been mated to a male of the other parental strain. The recipient female usually produced offspring both from her own and from the transplanted ova. The technique for transplanting ova was that described by Runner and Palm[10]. Altogether 157 operations for the transplantation of ova were completed. Twelve recipient females died; 41 lived but did not became pregnant; 40 lived, became pregnant, but did not produce any transplanted offspring; 64 lived, became pregnant, and produced one or more transplanted offspring. One litter of 10 contained one transplanted offspring that died before the sex and skeletal type were recorded, thereby making 63 the effective number of litters. Altogether 145 offspring, with sex and skeletal type recorded, were produced by transplanted ova, 106 in 43 litters from C57 ova and 39 in 20 litters from DBA ova. Unfortunately the DBA females gave a very poor yield of ova to be transplanted and the experiment had to be terminated before the planned number of 100 hybrid offspring from DBA ova was obtained.

TABLE I. Percentages of males and females with 25 presacral vertebrae (psv) in strains DBA/1JGn and C57BL/10JGn. Base numbers in parentheses

Strain	♂♂	♀♀	Total
C57	6.8 (310)	0.3 (309)	3.6 (619)
DBA	97.6 (211)	84.0 (194)	91.1 (405)

Results

In the C57 strain, about four percent have 25 psv; in DBA, about 91 percent have 25 psv

TABLE II. Percentages of mice with 25 presacral vertebrae (psv) in nontransplanted and transplanted hybrids of DBA/1JGn and C57BL/10JGn. Base numbers in parentheses

		Nontransplanted hybrids	Transplanted hybrids	Total
♀C57/♂DBA	♂♂	69.7 (185)	66.7 (51)	69.1 (236)
	♀♀	70.4 (179)	74.6 (55)	71.4 (234)
	Total	70.0 (364)	70.7 (106)	70.2 (470)
♀DBA/♂C57	♂♂	61.2 (98)	82.6 (23)	65.3 (121)
	♀♀	48.4 (97)	37.5 (16)	46.9 (113)
	Total	54.9 (195)	64.1 (39)	56.4 (234)
Combined	♂♂	66.8 (283)	71.6 (74)	67.8 (357)
	♀♀	62.7 (276)	66.2 (71)	63.4 (347)
	Total	64.8 (559)	69.0 (145)	65.6 (704)

(Table I). Males tend to have 25 psv more often than do females.

Of the nontransplanted hybrids gestated by C57 females, 70 percent had 25 psv (Table II). Of those gestated by DBA females, 55 percent had 25 psv. The difference between the two groups of hybrids, gestated by their own mothers, is significant ($\chi^2 = 14.17$; χ^2 (0.05,2) = 5.99). (This and the two χ^2's in the next paragraph each have two degrees of freedom because three classes, 25 psv, asymmetrical, and 26 psv, were used in comparing the two classes of hybrids.) In percentages of mice with 25 psv, both classes of nontransplanted hybrids are intermediate between the parental strains and both are slightly more like DBA than like C57 mice. The hybrids more nearly like DBA mice are those gestated by C57 mothers and sired by DBA fathers.

Slightly different results were obtained in the transplanted hybrids gestated by females of the BALB strain (Table II). Again about 70 percent of the C57 ova fertilized by DBA sperm produced mice with 25 psv. It appears that a change in the uterine environment did not affect these hybrids. The same cannot be said with certainty about the other class of hybrids produced by DBA ova fertilized by C57 sperm. In these, the distribution of vertebral types shifted so that, with 64 percent of the transplanted hybrids having 25 psv, the distribution is neither significantly different from the distribution of the nontransplanted hybrids of the same cross of which 55 percent had 25 psv ($\chi^2 = 2.77$; χ^2 (0.05,2) = 5.99), nor significantly different from the transplanted hybrids of the reciprocal cross of which 71 percent had 25 psv ($\chi^2 = 1.81$; χ^2 (0.05,2) = 5.99). It is obvious that the small number of successful transplantations of DBA ova has left the interpretation in doubt. We cannot say that the changed uterine environment has changed the hybrids of this cross nor can we say that it has not done so.

It is further obvious from the full results in Table II that the interpretation is complicated by the difference between males and females. In the pure strains, males tend to have 25 psv more often than do females. There is a similar difference in both the non-transplanted and transplanted hybrids from DBA ova (61.2 *v.* 48.4 and 82.6 *v.* 37.5 percent); there is essentially no difference between the sexes of the nontransplanted hybrids from C57 ova (69.7 *v.* 70.4 percent); but there is a difference in the opposite direction between the sexes of the transplanted hybrids from C57 ova (66.7 *v.* 74.6 percent).

Moreover, this tabulation of the results obscures the magnitude of the litter-to-litter variation and the possible dependence of the presacral vertebrae on litter size. The method of χ^2 clearly does not adequately compare the transplanted and the nontransplanted hybrids.

A much more adequate method consists of devising a metric for each litter in each of the four classes of hybrid progeny — reciprocal crosses, both transplanted and nontransplanted. More exactly, four metrics are needed, one to measure the litter size (L), one to measure the difference in numbers of males and females or a sex metric (S), one to measure the numbers of mice in the two classes of presacral vertebrae (V), and one for the association of sex and psv number (I). Once these are available, their means, variances, and covariances within and between classes may be computed; the effect of L on the variances of S, V, and I and the effect of S, V, and I on each other may be adjusted by the usual regression methods; and finally the significance of the differences between the reciprocal crosses in the

TABLE III. Means of the metrics for litter size (L), numbers of males and females (S), number of presacral vertebrae (V), and interaction of sex and psv (I) in nontransplanted and transplanted hybrids

		Number of Litters	L	S	V	I
Nontransplanted hybrids	♀C57/♂DBA	57	9.96	0.06	2.01	−0.06
	♀DBA/♂C57	42	8.96	0.07	0.31	0.48
Transplanted hybrids	♀C57/♂DBA	43	6.87	−0.09	1.14	−0.29
	♀DBA/♂C57	20	6.38	0.35	0.63	1.12
	Total	162	8.44	0.06	1.17	0.16

TABLE IV. Tests of significance for the difference between transplanted and nontransplanted hybrids, between reciprocal crosses, and their interaction. S = sex, V = presacral vertebrae, I = S × V, MS = mean square, df = degrees of freedom, F = the variance ratio, the critical value of which at the 5 percent level of significance for 1 and 158 degrees of freedom is F(0.05; 1, 158) = 3.90

	df	S		V		I	
		MS	F	MS	F	MS	F
Between transplanted and nontransplanted hybrids, A	1	0.14	—	2.64	—	1.54	—
Between reciprocal crosses, B	1	1.75	—	42.26	7.09	33.17	8.82
A × B	1	1.64	—	12.28	2.06	6.49	1.73
Within classes	158	3.40		5.96		3.76	

transplanted and the nontransplanted hybrids may be tested. The quantity of special interest is the one which measures the consistency of the differences in the adjusted V's between the reciprocal crosses in the transplanted and nontransplanted hybrids.

A suitable metric is one devised by Freeman and Tukey[1]. Briefly it consists of counting the number (n_i) of mice in each of the four categories in each litter:

n_1 = number of males with 25 psv,
n_2 = number of males with not-25 psv,
n_3 = number of females with 25 psv,
n_4 = number of females with not-25 psv.

Next each number (n_i) is transformed to a new scale (x_i) by a square root transformation,

$$x_i = \sqrt{n_i} + \sqrt{n_i + 1},$$

which for a Poisson-type variable with expectations near unity will stabilize the variances and permit variable effects to be additive. This transformation has been used previously for a comparable analysis and the arithmetical steps have been described[5].

It follows that:

$$L = x_1 + x_2 + x_3 + x_4,$$
$$S = x_1 + x_2 - x_3 - x_4,$$
$$V = x_1 - x_2 + x_3 - x_4, \text{ and}$$
$$I = x_1 - x_2 - x_3 + x_4.$$

For the nontransplanted hybrids, the L metric measures the litter sizes, but for the transplanted hybrids, it measures the number of successful transplantations of ova. Neither the number of fostered offspring nor their vertebral type was correlated with the number of native offspring.

The means of these four metrics are given in Table III. The litter size metric L is larger in nontransplanted than in transplanted hybrids and is larger when the litters are produced from C57 ova than when they are produced from DBA ova. The other metrics, S, V, and I are all mildly correlated with L and with each other, so their variances and covariances were adjusted by making L constant and then by making S, V, and I constant in turn.

The S metric which measures the difference in the numbers of males and females is small in each class and is of no further interest. The V metric shows that the difference between the nontransplanted hybrids is 1.70 (= 2.01 − 0.31) and between the transplanted hybrids is 0.51 (= 1.14 − 0.63). These two quantities should be alike if the transplanted and the nontransplanted hybrids are alike. The difference between them, 1.19 (= 1.70 − 0.51 = 2.01 − 0.31 − 1.14 + 0.63) is the quantity whose deviation from zero is in question. The analysis of variance of Table IV shows that in the case of V, the mean square which measures the difference between 1.70 and 0.51 is only 2.06 times larger than the error mean square and is not significant. On the other hand, the mean square which measures the difference between the reciprocal crosses, transplanted and nontransplanted combined, that is, the test of the significance of 2.21 (= 2.01 − 0.31 + 1.14 − 0.63), is 7.09 times larger than the error mean square and is highly significant. Furthermore the difference between the transplanted and the nontransplanted hybrids, measured by 0.55 (= 2.01 + 0.31 − 1.14 − 0.63) is not significant ($F < 1$). Therefore, it must be concluded that while there is a significant difference between the reciprocal crosses the difference in the percentages with 25 psv is about the same order of magnitude in both the transplanted and the nontransplanted hybrids.

The I metric, which measures the association of sex (S) and psv number (V) is likewise of interest. Tables III and IV show that the sex of the hybrid is associated with the number of presacral vertebrae in an unexpected way. The I metric tends to be negative (meaning fewer males with 25 psv and more females with 25 psv) in progeny from C57 ova and positive (meaning more males with 25 psv and fewer females with 25 psv) in progeny from DBA ova. The difference between the reciprocal crosses, transplanted and nontransplanted hybrids combined, is 1.95 (= − 0.06 − 0.48 − 0.29 − 1.12). The mean square which measures this difference is 8.82 times larger than the error mean square and so the difference is

TABLE V. The combinations of genomes, cytoplasms of the ova, and uterine environments in the four classes of transplanted and nontransplanted hybrids. C57 = C57BL/10JGn, DBA = DBA/1JGn, BALB = BALB/cJGn, psv = presacral vertebrae

		Nontransplanted		Transplanted	
		(1)	(2)	(3)	(4)
Genome of hybrid		♀C57/♂DBA	♀DBA/♂C57	♀C57/♂DBA	♀DBA/♂C57
Cytoplasm of ovum		C57	DBA	C57	DBA
Uterine environment		C57	DBA	BALB	BALB
Percent 25 psv	♂♂	70	61	67	83
	♀♀	70	48	75	38
	Total	70	55	71	64

Classes (1) and (2) differ both in cytoplasm and in uterine environment.
Classes (3) and (4) differ only in cytoplasm.

significant. This means that the male and female offspring of reciprocal crosses differ in the number of presacral vertebrae, but in opposite directions. However, there is no such difference between the transplanted and nontransplanted hybrids. The quantity which measures this difference, 0.41 (= − 0.06 + 0.48 + 0.29 − 1.12), is not significantly different from zero ($F < 1$). Further, the I metric shows a difference of 0.54 (= − 0.06 − 0.48) between the nontransplanted hybrids and of 1.41 (= − 0.29 − 1.12) between the transplanted hybrids. The difference of these two numbers, 0.87, is not significant ($F = 1.73$). This means that the opposite associations of sex and psv numbers detected between reciprocal crosses does not significantly change between transplanted and nontransplanted hybrids.

Discussion

While too few transplantations were accomplished with DBA ova to give a definitive experiment, the results summarized in Table V show: (1) that there is a patroclinous difference between the nontransplanted offspring of reciprocal crosses of the C57 and DBA strains; (2) that this difference is not significantly altered when the hybrids are grown in the uterine environment of the BALB strain; and (3) that the difference between the hybrids is different in magnitude in the two sexes.

Regardless of the sex differences, it is clear that the difference between the offspring of the reciprocal crosses is not reduced significantly by giving them a uniform uterine environment. In females the difference is, if anything, enhanced. This suggests that the difference between the hybrids, both transplanted and nontransplanted, is due to differences in the cytoplasm of the ova produced by the two strains. The direction of the difference leads to the conclusion that the cytoplasm of DBA ova tends to produce mice with more presacral vertebrae than does the cytoplasm of C57 ova. Since DBA mice ordinarily have fewer presacral vertebrae than do C57 mice, the cytoplasmic difference is in the opposite direction from the genetic difference. If the cytoplasmic difference could be removed, the difference between the two strains should be even larger than that observed.

The difference between the sexes may be thought of in two ways: (1) while the usual difference between the sexes occurs in the DBA ♀/C57 ♂ hybrids (fewer presacral vertebrae in males than in females) there is little or no difference between the sexes in the C57 ♀/DBA ♂ hybrids; or (2) while there is a striking difference between the female hybrids, there is a smaller difference between the male hybrids. Since little or nothing is known about the causes of the difference in number of presacral vertebrae between the sexes or between the offspring of reciprocal crosses there is at present no basis for choosing between these points of view. One possible explanation for these peculiar results is that the parental strains differ in sex-linked genes which affect the male hybrids in a direction opposite to that of the cytoplasm, the two effects nullifying each other. Without further tests, however, it would be premature to suppose that sex-linked genes have been demonstrated.

The conclusion that the difference between the offspring of the reciprocal crosses is due to the cytoplasm of the ova is not in agreement with the published results of other experiments in which transplantation has been used to study the effect of maternal environment on the number of presacral vertebrae. Runner[9] transplanted ova from strain 129 into BALB/c and found that the offspring had more presacral vertebrae than offspring from untransplanted ova. McLaren and Michie[8], in an experiment with offspring of reciprocal crosses between the C3H and C57BL strains in which there is a matroclinous difference, found that C3H ova fertilized by C57BL sperm and transplanted into C57BL females produced mice like the nontransplanted hybrids of C57BL ♀/C3H ♂. These experiments both suggest an effect of the uterine environment, and the experiment of McLaren and Michie, in addition, shows no effect of the cytoplasm of the ovum. These three experiments with transplanted ova are different in several respects. Runner compared transplanted and nontransplanted pure strain ova. McLaren and Michie compared transplanted and nontransplanted hybrids. Our experiment compares transplanted and nontransplanted hybrids of reciprocal crosses. Ova were fertilized before transplantation in McLaren and Michie's experiment, after transplantation in ours. In addition, different strains of mice are compared in the three experiments. It is possible that there is no single answer to the question of the source of the maternal effect. In some cases the hybrids may differ in the number of presacral vertebrae because of differences in the uterine environments, in others because of differences in the cytoplasm of the ova. It is clear that larger experiments are needed to reconcile these conflicting results and to identify the causes of differences between offspring of reciprocal crosses.

Summary

Offspring of reciprocal crosses of the DBA/1JGn and the C57BL/10JGn strains of mice differ in the number of presacral vertebrae. The difference is larger in females than in males and the hybrids tend to resemble the strain of the male parent. Transplantation of ova of each strain into females of the BALB/cJGn strain, followed by fertilization by sperm of the other strain to produce transplanted hybrids, did not significantly reduce the difference between the hybrids. The difference between the hybrids appears to be due to differences between the cytoplasm of the two kinds

of ova rather than to differences between the uterine environments provided by the mothers.

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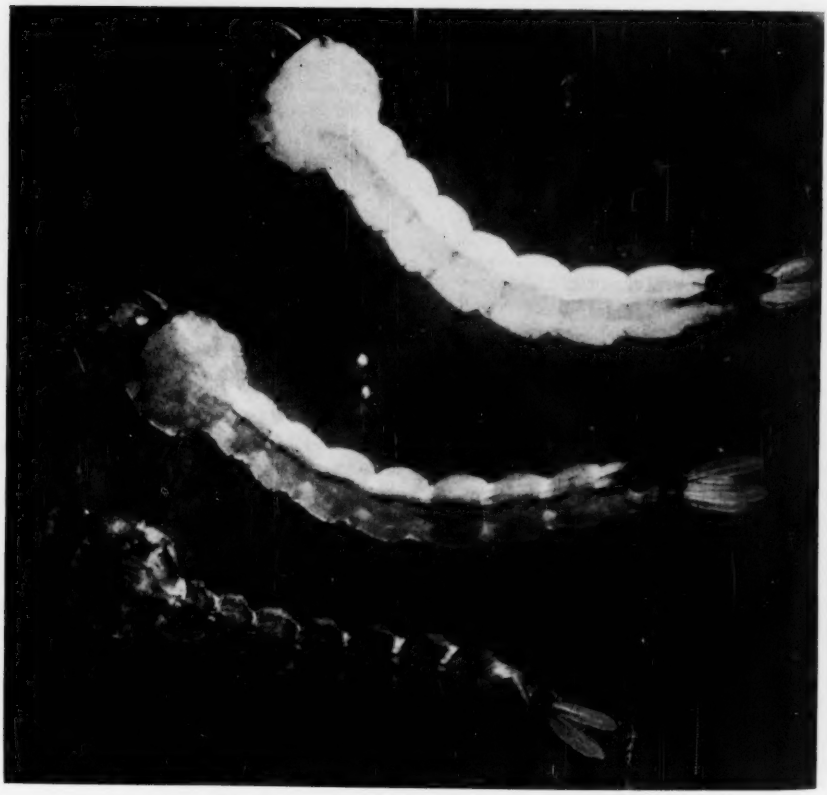
THE INHERITANCE OF LARVAL PIGMENTATION IN AEDES AEGYPTI

GEORGE B. CRAIG, JR., AND NICHOLAS W. GILLHAM*



COLOR FORMS OF MOSQUITO LARVAE
Figure 7

Fourth instar larvae of *Aedes aegypti* showing types of coloration caused by differences in storage of excretory products in the fat body. Top larva yellow (yy), lacking granules of uric acid. Middle larva wild type (y^+y^+), normal deposition of uric acid granules. Bottom larva melanotic (y^my^m), excess accumulation of uric acid.

IN laboratory cultures of *Aedes aegypti* there is a great deal of variation in larval coloration, but generally speaking the larvae may be grouped into three classes (Figure 7). The predominant class in most strains is dark in color, ranging from dark gray with a slight greenish cast to grayish brown. Larvae with this type of pigmentation are herein designated wild type or nor-

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mal. A second class is composed of pale yellow to yellowish white larvae. These are markedly less common than dark individuals in most strains. These two classes of color forms are mutually exclusive and can be segregated readily in cultures with sufficient food and uncrowded rearing conditions. The third class consists of melanotic larvae which are relatively smaller and appear dark brown to deep black. These are rarely encountered in most strains and segregation of black from dark gray is sometimes difficult. A study was initiated to determine the cause of these color differences, their hereditary basis and their frequency in strains of different origin.

The inheritance of larval pigmentation in mosquitoes has previously been studied only in *Anopheles quadrimaculatus* and the *Culex pipiens* complex. Coggeshall[1] has discussed the inheritance of white striping in larvae and pupae of *A. quadrimaculatus* and his results suggest a polygenic basis of inheritance for this character. On the other hand, most of the color characters investigated in *Culex* appear to be monofactorial. Huff[6] and Ghelelovitch[3] found monofactorial, autosomal inheritance of ground color in autogenous strains of the *Culex pipiens* complex, although the two pairs of alleles described by these authors appear to be unrelated. Huff described brown and green larvae with green resulting from the homozygous recessive condition while Ghelelovitch found gray-green to be dominant over yellowish brown. Spielman[11] crossed anautogenous individuals which bred true for pink larval fat with autogenous individuals whose larval fat was yellow. He concluded that pink is dominant to yellow, that this pair of alleles is autosomal and not linked to autogeny and that these alleles are not related to the sets described by Huff and by Ghelelovitch. Kitzmiller[7] described a melanotic condition in larvae of *Culex pipiens fatigans* which is apparently due to a three-factor recessive lethal. Laven and Chen[8], working with an autogenous strain of *pipiens*, have described a spontaneous mutant which acts as an autosomal recessive, and in the homozygous condition produces melanotic larvae which invariably die. They also demonstrated that the melanotic larvae become impoverished in their supply of free amino acids and peptides. It is interesting to note that none of the factors described in the above papers has been shown to be allelic.

Materials and Methods

Table I gives information on source of strains and available data concerning history of laboratory culture at the time when these strains were analyzed for larval pigmentation.

Larvae were reared in white enamel pans containing tap water and were fed powdered dog food on a regular schedule. Quantities of food were determined by the number and size of the developing larvae. Adults were fed either on slices of fresh apple or on cotton pads moistened with an aqueous solution of Karo syrup. Females were provided with anaesthetized guinea pigs as a source of blood meals.

The gross color differences between the various phenotypes are conspicuous to the naked eye and can readily be detected at some distance. Therefore, progeny counts requiring color segregation were made without recourse to microscopic equipment. Since the color differences disappear shortly after death, preserved larvae cannot be used to distinguish this character. The factors responsible for these gross color differences are best seen in living larvae compressed between a slide and cover slip and observed with the compound microscope.

All crosses were made with single pairs. To insure virginity in specimens used in crossing experiments, female pupae were placed in individual vials and adults were allowed to emerge in isolation. Mating, feeding and oviposition took place in cages made from pint cardboard ice cream containers. A shell vial lined with filter paper and filled with water was inserted through a hole in the bottom of the container to allow oviposition. Using these rearing techniques, inbred lines pure for the various color

types were established and used in genetic analysis. Inbred lines from single pair brother-sister crosses have been maintained for 20 generations for both yellow and normal types of larval pigmentation.

Late third and fourth instar larvae were used to determine color ratios since coloration is not as conspicuous in earlier instars. These larvae had to be reared under relatively uncrowded conditions (circa one larva per square centimeter of surface area of the medium) since overcrowded larvae all tend to be light.

Results

Nature of pigmentation

Most of the color variation is due to differences in deposition of pigment in the cells of the parietal fat body. These cells form a thin, single layer just beneath the transparent epidermis and are abundantly distributed throughout the thorax and abdomen. In the more common (presumably normal or wild type) dark larvae, numerous opaque granules are deposited in the cells of the fat body, giving the larva a grayish opaque appearance. DeBoissezon[2] describes such granules in larvae of *Culex pipiens* and gives chemical evidence as to their purine nature. No such granules are found in the yellow type of *A. aegypti*. Hence, the fat body of the latter appears translucent and the larval body appears yellow to white on gross examination. On the other hand, the melanotic appearance of very black larvae is caused by an excess deposition of granules.

These granules are probably concretions of uric acid as described by Wigglesworth[14] in his study of various materials in the fat body of *Aedes aegypti*. When normal larvae are

TABLE I. Source of strains of *Aedes aegypti* examined for larval pigmentation

Designation	Source	Strain history
NIH	P. A. Woke National Institutes of Health Bethesda, Maryland	Laboratory rearing at least 20 years.
FD	N. W. Gillham Chemical Corps Biol. Labs. Fort Detrick, Maryland	Laboratory rearing at least 20 years.
TEX	D. W. Micks School of Medicine University of Texas Galveston, Texas	Laboratory rearing at least 15 years.
GER	H. Laven Max-Planck-Institut für Biologie Tübingen, Germany	Laboratory rearing at least 15 years.
TRIN	R. W. Fay Technical Development Labs. U. S. Public Health Service Savannah, Georgia	Collected in Trinidad, B.W.I., 1954. Reared in laboratory 3 years. Resistant to DDT.
TRIN-HR	G B. Craig Chemical Warfare Labs Army Chemical Center, Md.	From TRIN strain, lab selection with DDT for 13 generations. Very high resistance.
KUALA	W. W. Macdonald Institute for Medical Research Kuala Lumpur, Malaya	Laboratory rearing at least 3 years.
KLANG	W. W. Macdonald Klang Island, Malaya	Recent field collection, 2 generations in lab before analysis.
PHIL	L. La Motte Manila, Luzon Philippine Islands	Recent field collection, 1956. Reared several generations at Ft. Detrick, Md.
QUEEN	P. C. C. Garnham London School of Tropical Medicine and Hygiene London, England	Laboratory colony several years, originally from Saudi Arabia. This is *A. aegypti* var. *queenslandensis*.

fixed in aqueous Bouin's, washed in alcohol and mounted in euparal, small sheaves of needle-like crystals replace the granular structures observed in living larvae. These crystals are similar to those illustrated by Wigglesworth. When stained with carmine and methylene blue using the Schultz-Schmitz stain for sodium urate (Gurr[5]), the crystals appear deep greenish blue, characteristic of uric acid.

Granules are deposited evenly throughout the fat cells in newly-molted fourth instar larvae but older individuals show an accumulation of granules around the periphery of the cells. When larvae are starved, the granules become more conspicuous, due to accumulation of waste material and to disappearance of the fat globules which may conceal the granules. On gross examination, the fat body of a mature larva appears much darker toward the posterior margin of each segment. This is probably caused by a thickening of the fat body in these regions which results in more abundant granules. Granules have not been observed in newly hatched larvae but are present in young second instars and pigmentation becomes increasingly conspicuous thereafter.

A secondary means of differentiating the color classes is provided by the degree of pigmentation of the sclerotized regions. The head capsule, air tube and anal saddle of the wild type larva are always darker than the corresponding parts of the yellow phenotype. The developing pupal air trumpets, observed through the thoracic cuticle, also show this variation. Since the sclerotized regions show progressive darkening after molting and since this color variation is relative rather than absolute, it is not as satisfactory for purposes of classification as is color of the fat body. As would be expected, the sclerotized areas of the melanotic form appear darker in color.

Color differences are also found in the pupae. The constant darkening of the cuticle during the pupal stage makes segregation difficult. However, at any given age, pupae from yellow larvae are lighter than those from normal larvae.

No external indication of variation has been observed in adults from these strains. However, when the fat body of aged females is dissected out, granules are conspicuous in individuals from normal and melanotic larvae but not from yellow larvae. This difference is evident but less pronounced in males and in younger females.

Rearing conditions have considerable influence on the viability and vigor of the various phenotypes. Under ideal conditions, the yellow phenotype appears to have few adverse effects. If food supplies are appropriate and sufficient surface space of the rearing medium is allotted,

TABLE II. Segregation of larval color in the F_2 from single-pair crosses between inbred lines of wild type and yellow larvae

Parental cross	F_1 phenotype		F_2 phenotype		χ^2	Fails to fit 3:1 ratio at 5% level
	wild type	yellow	wild type	yellow		
yellow ♀ × wild type ♂	54	0	73	41	7.301	x
			89	29	0.011	
			46	10	1.529	
	41	0	214	80	0.766	
			41	22	3.307	
			66	18	0.571	
	99	0	211	68	0.058	
			43	7	3.227	
			41	15	0.095	
	165	0	68	29	1.241	
			19	15	6.628	x
wild type ♀ × yellow ♂	109	0	209	87	3.045	
			84	28	0.000	
			161	89	14.967	x
	122	0	474	194	5.820	x
			37	21	3.891	x
			82	22	0.821	
	138	0	212	88	3.004	
			11	4	0.022	
			33	16	1.531	

mosquitoes from the yellow and normal stocks are equal with respect to rate of development, size, longevity and fecundity. However, when short of food or when overcrowded, the growth rate of yellow larvae is reduced far below that of normal larvae. When given excess food, normal larvae are able to inhibit scum formation, but yellow larvae appear unable to do so and often die in the fouled medium. Death most often strikes freshly molted pupae. No tanning occurs and the dead forms are an opaque white with a pinkish cast around the eyes. Melanotic larvae show a similar inability to cope with adverse rearing conditions.

Melanotic larvae lack vigor under the best conditions. They develop slowly, sometimes requiring a month to pupate rather than the usual week. Frequently, such slow-pupating larvae are covered with heavy infestations of *Vorticella*, which take advantage of the slow development to attach to the larval cuticle. If larvae with *Vorticella* are able to pupate, they usually emerge successfully as adults. In the course of the present work, most of the melanotic larvae isolated from natural populations died before pupation and this factor was thought to be lethal. However, after attempts at rearing several hundred melanotic larvae failed, a few managed to complete the cycle and from these few a melanotic strain was established. Adults of this strain were small and frequently sterile. Females did not engorge fully when taking a blood meal and produced few eggs. Males were sluggish and showed reduced sexual activity. Thus, this characteristic is semi-lethal.

Several other shades of color have also been observed although these have not been subjected to close analysis. Normal larvae of the KUALA and KLANG strains appear brownish to tan when compared with the grayish cast in wild type larvae of other strains. The brown color is also caused by fat body granules. On microscopic examination, these granules appear very similar to those found in wild type larvae.

Inheritance of larval pigmentation

To analyze the factor responsible for yellow larva, separate strains of dark, wild type larvae and yellow larvae were isolated and inbred for six generations. These strains bred true for their respective colors. Four single-pair crosses were made with inbred yellow females and wild type males. Three reciprocal crosses were also made. In every case, the F_1 individuals were all dark, wild type (see Table II). These results have been duplicated many times.

From the F_1 of each of the original crosses two or three brother-sister crosses were made. The color ratios of F_2 progenies are shown in Table II together with χ^2 values derived from an expected ratio of wild type to yellow of 3:1. Crosses failing to fit the expected ratio at the 5 percent level of confidence are marked with an x. As seen in this Table, five out of 20 crosses failed to fit the hypothesis. In no case was the departure extreme.

As a further check, F_1 females and F_1 males were backcrossed to inbred yellow males and females. The results of these crosses are summarized in Table III. Three out of 17 crosses failed to fit the expected 1:1 ratio of wild type to yellow at the 5 percent level of confidence. Again, no extreme departures were observed.

Out of the 37 crosses, all of the eight which failed to fit the expected ratio showed disproportion in favor of yellow. Subsequent investigation has indicated that heavy overcrowding or underfeeding will sometimes produce yellow phenotype among populations

TABLE III. Segregation of larval color in the backcross from single-pair crosses between inbred lines of wild type and yellow larvae

Cross*	Backcross larvae: wild type	Backcross larvae: yellow	χ^2	Fails to fit 1:1 ratio at 5% level
F_1 ♀ (yellow ♀ × wild type ♂) × yellow ♂	17	20	0.243	
	28	45	3.959	x
	39	25	3.061	
	47	38	0.953	
	81	84	0.054	
	47	58	0.867	
yellow ♀ × F_1 ♂ (yellow ♀ × wild type ♂)	22	17	0.642	
	20	20	0.000	
	127	156	2.971	
	174	196	1.308	
	146	196	7.530	x
F_1 ♀ (wild type ♀ × yellow ♂) × yellow ♂	23	47	8.229	x
	54	42	1.500	
	129	136	0.185	
yellow ♀ × F_1 ♂ (wild type ♀ × yellow ♂)	97	121	2.642	
	8	14	1.636	
	38	53	2.473	

*F_1 parental types indicated in parentheses

heterozygous for yellow. The results of the eight crosses which failed to fit were probably influenced by the production of yellow phenocopies in heterozygotes. It is of interest that such phenocopies have been produced readily in heterozygotes but have not been observed in individuals homozygous for the wild type factor.

The results of the remaining 29 crosses confirm the hypothesis that wild type and yellow larval coloration are caused by a single pair of autosomal alleles with wild type being dominant to yellow. These alleles are here designated $y+$ for normal pigmentation and y for yellow. It should be noted that the dominance of wild type is not complete. Heterozygotes (y $y+$) usually appear somewhat lighter than homozygotes ($y+$ $y+$), although these slight differences in shading are difficult to separate on an absolute basis.

To test for allelism, several crosses were made between homozygous individuals from different strains. These crosses are summarized in Table IV. In all cases, progeny produced by parents having yellow larvae were yellow. This demonstrates that the same set of alleles for larval color is present in the GER, FD, NIH, TRIN, KUALA and QUEEN strains.

TABLE IV. Results of crosses between adults produced from yellow larvae of different strains of *Aedes aegypti*

Cross	Color of F_1 larvae: wild type	yellow
GER female × FD male	0	71
	0	77
FD female × GER male	0	60
GER female × NIH male	0	51
	0	28
NIH female × GER male	0	94
	0	78
	0	21
GER female × TRIN male	0	57
GER female × KUALA male	0	88
	0	40
QUEEN female × GER male	0	87
	0	64

In order to determine the mode of inheritance of the factor causing melanotic larvae, another set of crosses was made. Single females from an inbred melanotic strain were crossed with single males from an inbred normal strain and reciprocal crosses were made. Results of this series were somewhat more difficult to interpret because the pigmentary differences were matters of degree rather than presence or absence. While most melanotic larvae could never be confused with normal types, a few showed some reduction in pigmentation approaching the darkest part of the range of expression found among wild type individuals. In general, however, the F_1 progeny were all of wild type color. The F_2 showed about one quarter melanotic forms and a test backcross to wild type produced melanotic and wild type progeny in approximately a 1:1 ratio. Based on these results, it appears that the melanotic condition is caused by an autosomal factor which is recessive to the factor for normal pigmentation. Once again, dominance of wild type is not complete. The F_1 heterozygotes appeared slightly darker than the wild type but much lighter than melanotic.

To test for allelism with y, crosses were made between melanotic and yellow individuals. As indicated in Table V, the F_1 progeny of such crosses were wild type and did not resemble either parent. The F_2 progeny, on the other hand, showed a segregation in good agreement with one melanotic to two wild type to one yellow. These results are consistent with the hypothesis that yellow and melanotic are allelic factors. On this basis, melanotic can be tentatively designated as y^m. An equally likely alternative hypothesis is that y and y^m are non-allelic. Di-hybrid inheritance with close linkage would also account for these results. Extensive crossover tests will be required to prove or disprove allelism.

The brown color observed in the Malayan strains behaved like wild type when crossed with yellow. Ratios of 1:1 in the backcross and 3:1 in the F_2 of crosses between brown and yellow larvae were obtained. Crosses between brown and wild type were difficult to

TABLE V. Segregation of larval color in the F_2 from single-pair crosses between inbred lines of melanotic and yellow larvae

Parental cross	F_1 phenotype*: melanotic	wild type	yellow	F_2 phenotype*: melanotic	wild type	yellow	χ^2 for 3:1 yellow to non-yellow
melanotic ♀ × yellow ♂	0	63	0	36	68	34	0.008
				36	71	30	0.703
				59	112	45	2.000
	0	24	0	47	102	60	1.533
	0	47	0	----	----	----	
yellow ♀ × melanotic ♂	0	82	0	30	57	34	0.619
	0	17	0	----	----	----	
	0	31	0				

*Separation of melanotic from wild type not positive

interpret because of intergrading, intermediate forms in the progeny.

Frequency of phenotypes

To determine phenotypic frequency, ratios of yellow and wild type individuals were estimated for all of the strains mentioned. As indicated in Table VI, the wild type phenotype predominated in most strains. However, there was almost a 1:1 ratio in the strain from the Philippines and the laboratory strains from Germany (GER) were 100 percent yellow, possibly indicating that GER may have originated from variety *queenslandensis*. Other morphological characteristics of the GER strain bear out this hypothesis.

A comparison of phenotypic percentages was made between a segment of the Trinidad strain which had been selected for high resistance to DDT for 13 generations (TRIN-HR) and the original Trinidad strain (TRIN) which was not selected. No significant change occurred in the ratio of yellow to wild type during the period of selection for resistance. Thus, it appears that neither phenotype has adaptive significance with regard to DDT-resistance in this strain.

Larvae of other species of *Aedes* were examined for the yellow phenotype. Material analyzed included *Aedes albopictus, A. pseudoscutellaris, A. polynesiensis, A. atropalpus* and *A. vexans*. Larvae of all of these species were dark and had conspicuous pigment granules in the fat body. However, although several thousand individuals of each species were examined, the yellow phenotype was not observed.

Melanotic larvae were found in several strains including TRIN, KUALA and NIH. In no strain were they abundant. The frequency of the phenotype was well below 0.1 percent. The melanotic line eventually colonized came from TRIN and attempts at rearing melanotic forms from the other strains were unsuccessful.

The brown color found in the KUALA and KLANG strains was apparent in all of the non-yellow larvae and this color is considered as wild type in Table VI.

Discussion

A number of recent authors have emphasized the scarcity of genetic information on mosquitoes. Kitzmiller[7] pointed out the need for genes with clear-cut morphological effects which could be used as markers and testers. More recently, Mattingly[10] has indicated that no simple, monofactorial, *Drosophila*-type characters are known for *Aedes aegypti*. It would appear that the yellow and melanotic forms of larval pigmentation described herein will be of value as genetic markers. Yellow larva has been used effectively as a chromosome marker in studies of multiple fertilization and mating behavior (VandeHey and Craig[13]).

Its conspicuous phenotype, high expression and simple mode of inheritance make the gene for yellow larva a good base for construction of linkage maps. To date, this character has been used to determine linkage relationships of a number of other genes (to be published elsewhere). *Aedes aegypti* has but three pairs of chromosomes and yellow larva is not sex-linked. Since this is the first autosomal gene described for this species, the chromosome on which yellow larva occurs is here designated as 2.

It seems evident that the granules which cause pigmentation are purines stored as excretory products within the cells of the fat body. This pigmentation probably does not correspond to the variations in the color of the sclerotized regions of larvae of *Aedes aegypti* described by Golberg and DeMeillon[4]. In the latter case, degree of cuticular darkening of the head capsule and air tube was dependent upon the quantity of phenylalanine and tyrosine available in the larval diet. No mention was made of effects of amino acid deficiencies on the fat body or on the unsclerotized cuticle which covers most of the body. On the other hand, De Boissezon[2] and Wigglesworth[14] clearly describe histochemical analysis of granules in the fat body and their results are consistent with the observations reported herein. A positive demonstration that these granules are uric acid and not related purine compounds has yet to be made.

These granules are accumulated in increasing quantities during larval and pupal development but are less common in young adults than in older ones. Terzian *et al.*[12] have shown that newly emerged adults of *Aedes aegypti* excrete considerable amounts of uric acid. They found that the output of uric acid in excreta decreases rapidly thereafter and remains at a low level during adult life, so long as the mosquitoes are maintained on a protein-free diet. These workers indicate that the newly emerged mosquitoes are excreting products of pupal metamorphosis rather than products of adult metabolism. On

TABLE VI. Distribution of the yellow phenotype among larvae of several strains of *Aedes aegypti*

Strain	Percent		Total larvae
	wild type	yellow	examined
Laboratory			
KUALA	99.0	1.0	979
NIH	95.1	4.9	1,372
TRIN	93.8	6.2	770
TRIN-HR	93.7	6.3	1,027
FD	70.5	29.5	1,021
TEX	64.6	35.4	820
GER	0.0	100.0	>1,000
QUEEN	0.0	100.0	>1,000
Wild			
PHIL	51.6	48.4	543
KLANG	92.6	7.2	1,006

this basis it seems probable that the uric acid granules stored by the immature stages are released by histolysis of the fat body during metamorphosis and are subsequently eliminated by excretion of the newly emerged adult. However, protein metabolism obviously continues during adult life and hence, more granules are accumulated in the fat bodies of older adults.

An analysis of the nature of the alternate excretory pathways utilized by yellow larvae should be of considerable interest. Storage excretion of excess wastes would seem to be a rather fundamental process for mosquito larvae. Their growth and development may be too rapid to allow normal elimination of all wastes. In yellow larvae, it would appear that either waste materials are stored as some purine less conspicuous than uric acid or that all wastes are excreted and none accumulate in the fat body. Biochemical analysis of the larval fat bodies of various color types should answer this problem.

As mentioned earlier, Terzian *et al.*[12] described uric acid patterns in the excreta and body tissue of adult *Aedes aegypti.* Their analysis was based on pools of mosquitoes selected at random from a standard laboratory colony without regard to larval pigmentation. On the basis of the strains examined in the present work, one would expect that their colony would contain both yellow and wild type larvae. Moreover, it seems likely that adults of these phenotypes would show very different patterns of uric acid excretion. It would be interesting to repeat their experiments using stocks pure for the three types of uric acid excretion described in the present work.

These mutations affecting pigmentation provide promising material for physiological studies of gene action. On the basis of phenotypic observation, it seems likely that the amount of pigmentation produced depends upon the type of alleles present, the gene dosage and the environment of the developing larva. Chemical assay of uric acid content of the various genotypes may indicate a direct relationship between the type of genes present and the quantity of uric acid produced. This would rule out simple dominance of the wild type gene over its alleles. Such a situation is better explained by incomplete dominance and an additive effect of genes for uric acid production. If one assumes that y and y^m are allelic, a series with progressively darker phenotypes would then show the following genotypes: yy (albino), $yy+$ (light normal), $y+y+$ (normal), $y+y^m$ (dark normal), y^my^m (melanotic). Observations to date are in accordance with this hypothesis.

The production of yellow phenocopies in individuals heterozygous for yellow ($yy+$) deserves further investigation. Under environmental stress such as overcrowding or improper feeding, the mechanism for storage of uric acid appears to be blocked. A careful analysis of specific environmental factors might provide information on genic activity in promoting uric acid production.

It is entirely possible that there is a whole series of alleles for pigmentation. The types described herein may represent only the extremes (y and y^m) and the mid-point ($y+$) of this series. The brown color observed in the Malayan strains may represent a fourth allele in this series (y^b). Other strains also show variation from the wild type color, appearing somewhat lighter or darker gray on the average. Segregation of these alleles by eye may prove impossible because of the range of individual variation found even within inbred strains.

Further studies of the geographic distribution of the various pigmentary types should be conducted. Most of the strains analyzed so far are from laboratory colonies and these data provide little information about variation in natural populations. However, based on its wide distribution and high frequency in some strains, yellow larva must have considerable adaptive value.

It is particularly interesting that variety *queenslandensis* is homozygous for yellow larva. This variety is known as the "pale form" of *Aedes aegypti* because the abdominal and mesonotal scales of the adults show extensive bleaching. In some specimens, the dorsum of the abdomen appears entirely white rather than black with white bands and the basic mesonotal color is golden rather than dark brown to black. Since both adults and larvae show marked albinism, it would appear that reduction in pigmentation is generally advantageous in the *queenslandensis* environment. Mattingly[9] gives evidence from several authors indicating that var. *queenslandensis* is entirely a domesticated form, being found primarily in and around houses. On the other hand, the darker forms of this species are more characteristic of natural habitats. Light color may well be adaptive in the domestic habitat. Laboratory experiments with population cages might be used to demonstrate the likelihood of this hypothesis.

In studies of tolerance to toxicants, behavior or physiology of *Aedes aegypti,* it is commonly assumed that "standard" laboratory colonies are homogeneous and reasonably homozygous because such colonies have been maintained under laboratory conditions for many years. As Mattingly[10] points out, this is not necessarily evidence for genetic homogeneity since apparent genetic stability may mask a balanced heterogeneity. Certainly, yellow larva shows balanced polymorphism, being found in varying frequencies in strains under laboratory culture for many years. The genetic stability of yellow larva is demonstrated in the line of the TRIN strain which was placed under selection with insecticides. After 13 generations of intense selection with DDT, there was no change in frequency of yellow larva. It would appear

that workers interested in standardized results might pay more attention to genetic composition of their strains, perhaps working with reasonably pure lines produced by repeated generations of single-pair, brother-sister matings.

Summary

Larvae of *Aedes aegypti* can be segregated into three classes based on body color: dark gray to yellowish brown (wild type), pale yellow and albinistic (yellow), blackish (melanotic). Normal color is caused by the deposition of dark granules of uric acid or a related substance in the fat body while the yellow phenotype is produced in the absence of these granules. These granules represent storage excretion of purines and are found within the cells of the fat body of pupae and adults as well as larvae. Viability and vigor of the yellow phenotype is comparable to the wild type under good rearing conditions but under adverse conditions, the yellow form is less successful. The melanotic type is sublethal.

Separate strains of wild type, yellow and melanotic larvae were isolated and inbred. All strains bred true for their respective colors. Crossing experiments indicate that yellow and melanotic are produced by single, autosomal factors which are recessive to wild type, the latter factor showing incomplete dominance in the heterozygous state. These factors may be part of a larger allelic series of factors for larval pigmentation. Alternatively, yellow and melanotic may be closely linked. Yellow phenocopies can be produced by overcrowding or improper feeding of larvae heterozygous for the yellow factor.

Among nine strains examined, the frequency of the yellow phenotype varied from 1 to 100 percent. *Aedes aegypti* var. *queenslandensis* was homozygous for yellow larva. Yellow larva had no adaptive value in a strain under selection with DDT. Melanotic larvae were uncommon, being found in only three strains with a phenotypic frequency below 0.1 percent.

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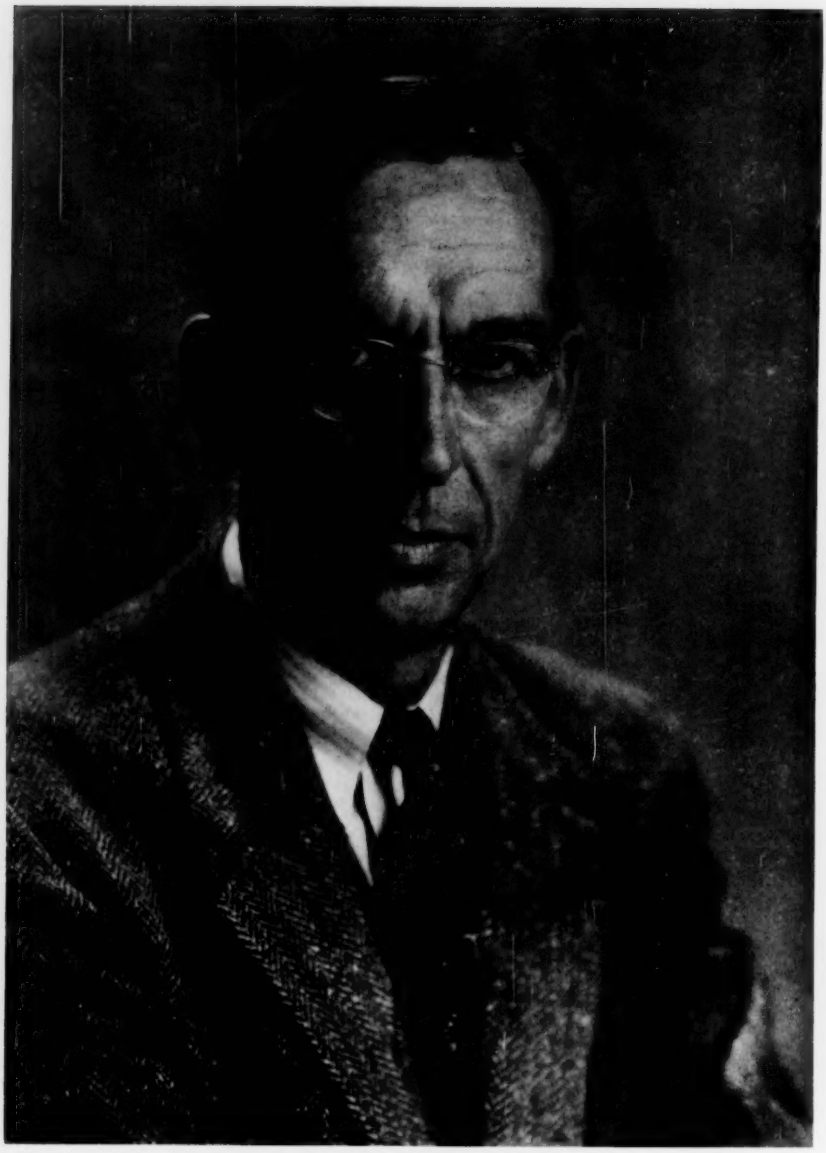

KENNETH WILLIAM NEATBY

1900-1958

KENNETH WILLIAM NEATBY

1900 - 1958

FOR almost 20 years before his untimely death on October 27, 1958, Kenneth W. Neatby was one of the most powerful influences in agricultural science in Canada. He left his mark on all facets of scientific agriculture: on scientific plant breeding through his early work; on teaching and extension in his middle years; and on research administration in the last decade of his life.

Dr. Neatby was born at Sutton, Surrey, England on March 30, 1900. His family moved to Canada in 1906 and settled at Earl Grey, Saskatchewan, where his father, Dr. A. M. Neatby, practised medicine for two years before homesteading near the town of Watrous. The period from 1908 to 1919 was spent on the farm. From 1920 to 1926 he studied and worked at the University of Saskatchewan where he obtained the degrees of B.S.A. in 1924 and M.S.A. in 1926. Further study at the University of Minnesota, where he specialized in genetics and plant breeding, obtained him a Ph.D. in 1931.

During the years from 1926 to 1935 he carried on intensive research on genetics and plant breeding. In the spring of 1926 he was appointed to the staff of the newly founded Dominion Rust Research Laboratory at Winnipeg and was placed in direct charge of breeding rust resistant varieties of common wheat. To this task he brought enthusiasm and energy, and it was due chiefly to his work that Renown, one of the first two Canadian varieties resistant to rust, became available to farmers in 1937, to be followed by the variety Regent two years later. From the beginning of this work, the mechanism of inheritance of rust resistance was a major interest. In conjunction with C. H. Goulden and J. N. Welsh he demonstrated that the mature-plant resistance so important in wheats with the H44 type of reaction was controlled by a single major gene conferring resistance to numerous stem-rust races, and that this type of resistance was inherited independently of the seedling reaction to the same races. Studies on the inheritance of resistance to individual races and groups of races were pursued during his stay at Winnipeg and during the year 1933-34 spent at Cambridge, England, on a fellowship from the National Research Council (U. S.), where he explored the genetic relationship between resistance to stem rust and several other diseases of wheat. It is of passing interest that his work at Cambridge brought him into close and friendly contact with Sir Rowland Biffen, the first man to study the inheritance of rust resistance. His interests, however, were not confined to the genetics of rust resistance. Among other studies may be mentioned his discovery of the virescent gene, *v*, the first gene for chlorophyll deficiency reported in the 42-chromosome wheats, later located by Sears in chromosome 3. Among his hobbies during the years at Winnipeg was the taxonomy of higher plants, which resulted in the formation of a herbarium at the Rust Research Laboratory.

In 1935 he was appointed head of the Department of Field Crops at the University of Alberta. From this time onward his personal influence came to be felt in ever increasing measure in various fields of scientific agriculture. He encouraged fundamental research and introduced adequate biometric study to the students of the Faculty of Agriculture of the University of Alberta. In 1940, he resigned his professorship to organize the Farm Service of the North-West Line Elevators Association, a group of grain companies desirous of developing an extension service for prairie farmers. It is a tribute to the soundness of his organization of this service that it still proceeds on the lines established by him, in 1940. It was during this period that

he was president of the Canadian Society of Technical Agriculturists (1943-44) and it was during his incumbency that its policies were reorganized and its name changed to the Agricultural Institute of Canada, an organization now comprising nearly all agricultural scientists and technologists in Canada.

The final period of his career opened with his acceptance, in 1946, of the post of Director of Science Service, Canada Department of Agriculture, Ottawa, the branch concerned with the principal scientific services of the Department. Having passed through the depression of the thirties and the Second World War with a minimum of expenditure on staff, equipment, and accommodation, the scientific services were sadly in need of attention. The task of expanding the services and providing them with adequate equipment and working quarters was immediately undertaken. Between 1946 and 1958 the annual budget was raised from less than three million dollars to more than ten million. Fundamental research was augmented by making departmental funds available to universities for carefully selected extramural research projects. These facts, and the splendidly equipped new Science Service laboratories, erected or in process of erection from coast to coast, testify to the success of Dr. Neatby's efforts. But he well knew that the quality and morale of the investigator was even more important to scientific success than fine laboratories and equipment; and an equal amount of effort was successfully spent on improving salaries and attracting able scientists to the service.

For several years Dr. Neatby had dreamed of a reorganization of the entire research work of the Department—a reorganization in which the artificial separation of Science Service and Experimental Farms Service would be eliminated. It was only recently that circumstances made this reorganization possible. Though, regrettably, he died before he could guide the newly reorganized services, of which he had been appointed head, it is some consolation to realize that he knew that his last efforts were crowned with success.

As was fitting, Dr. Neatby received many honors for his contributions to science and agriculture, among which may be mentioned honorary degrees from the universities of Saskatchewan (LL.D., 1948) and Manitoba (D.Sc., 1956), fellowships of the National Research Council (U. S.) (1933), the Royal Society of Canada (1947), and the Agricultural Institute of Canada (1949).

To science and agriculture it is the achievement that matters; to all those who knew him personally it is principally the man himself, characterized by the warmth of his personality, his wit and his sense of humor, the intelligence and charm of his conversation which made him the center of attention at all gatherings. His interests were remarkably wide. In his college days he was the outstanding actor at the university and president of its Dramatic Society. He possessed a magnificent bass voice which won him distinction at the Winnipeg Musical Festivals and elsewhere: at one time he seriously considered a professional singing career. To all this was added an intense joy of living which he communicated to others in his presence.

Dr. Neatby is survived by his wife, Janet, and one son, Andrew Mills, aged 9.

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HEREDITARY EXENCEPHALY IN THE FOWL

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DURING the 1954 hatching season, mild to severe exencephaly was noted in chicks hatched from six S. C. White Leghorn dams of the University strain. All affected chicks were sired by two half-brothers, and the two sires and six dams shared common ancestry, which suggested that the trait was hereditary. Exencephalic chicks were also produced in breeding tests during the three following years.

Description

There was a wide range in expression of the exencephaly, varying from a scarcely noticeable hydrencephalocele in some chicks to massive herniation of the brain in others. A moderately severe case is shown in Figure 8. The basic defect was cranioschisis, with the protrusion of brain parts apparently secondary. In mild cases the skin remained intact over the herniated area, but in severe cases there was no cutaneous covering. No other morphological abnormality was apparent, except in one very severe case in which microphthalmia was present. In most instances the cranial defect was in the fronto-parietal region, allowing extrusion of a portion of the cerebrum, but in some severely affected chicks the occipito-parietal portion was also patent, with herniation of both cerebrum and cerebellum through one large opening.

Throughout the study, all unhatched eggs were broken out and dead embryos were classified as either exencephalic or normal. (This classification could be made readily in embryos incubated 6-7 days or more.) Since the proportion of exencephalic cases was no greater in dead embryos than in hatched chicks, apparently the condition had no prenatal lethal effect. It did prove fatal, however, to most affected chicks during the first few days after hatching. Most such deaths appeared to be due to mechanical injury and cannibalism, with considerable hemorrhage from the susceptible tissues of the head. It was possible to rear a few exencephalic birds to maturity, including some with fairly severe expression of the trait. At one time (October, 1956), five mature females which had shown some degree of exencephaly as chicks were sacrificed for study of the

EXENCEPHALIC CHICK

Figure 8

This chick was typical of many with moderately severe hereditary exencephaly. It hatched normally, and no other morphological abnormalities were apparent. (*Photo by M. S. Cover*).

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PERMANENT CRANIOSCHISIS IN EXENCEPHALIC FOWLS

Figure 9

The basic cranial defect persisted in some chickens which were reared to maturity. Other apparent abnormalities are artifacts incurred during handling. (*Photo by J. H. Vondell*).

skulls. In three of the five, there was no remaining indication of cranioschisis, at least as observed grossly; but in the other two, gross evidence of the basic defect was still present (Figure 9). Mild ataxia was evident in some of the exencephalic birds which were reared successfully.

Breeding Tests

1954-55

The exencephalic chicks produced in 1954 and 1955 were the progeny of certain phenotypically normal dams mated to two normal half-brothers, and no such chicks resulted from any other of the many pedigreed matings carried out during those years. A further indication that the condition had a genetic basis was the fact that the incidence of exencephaly in the progeny of these carriers was much higher than has ever been reported for similar non-genetic teratological traits. Of all classifiable chicks (hatched chicks plus dead embryos), 8.9 percent were exencephalic, with a range of 4.1 percent to 17.6 percent for individual matings. The results of these carrier matings are summarized in Table I.

1956

Two exencephalic cockerels were available. One of these was mated to the only two original carrier females still alive (mating A); the other was mated to two exencephalic pullets (mating B). From mating A, one hen (#817) pro-

TABLE I. Incidence of exencephaly in 1954-55 progeny of normal carriers, expressed as number exencephalic/total number classifiable

♂ # \ ♀ #	605	690	817	819	1016	1046	Sire totals
739	4/46	6/63	3/19	0/13	1/17	0/0	14/158
1041	0/13	0/9	6/32	5/45	1/32	3/37	15/168
Dam totals	4/59	6/72	9/51	5/58	2/49	3/37	Grand total 29/326

duced nine herniated chicks in a total of 55 (16.4 percent); the other hen (#1016) produced no affected chicks in 19. From mating B, one pullet produced 18 exencephalic offspring in a total of 63 (28.6 percent); the other produced one in 61 (1.6 percent), (Table II.).

1957

Two exencephalic males (not the two used in 1956) were used in a diallel mating scheme with four similar females. One of these females produced no chicks at all, and one of the others produced all normal chicks. Progeny of the other two exhibited 21.7 percent and 33.3 percent exencephaly, respectively, (Table II).

1958

The matings in this year were designed to test the apparent recessiveness of the exencephaly. In one pen, two exencephalic males were used in diallel matings with 11 normal females of an unrelated S. C. White Leghorn strain. In the other pen, two normal males of the unrelated strain were used in diallel matings with seven herniated females. Of 415 classifiable chicks produced (those hatched plus dead embryos), none was exencephalic.

Discussion

The exencephaly described here is obviously inherited, but the underlying genetic mechanism is obscure. The defect may be caused by a recessive gene with incomplete penetrance, but the data could support other possible interpretations: multigenic inheritance may well be involved. Possibly the exencephalic birds are *phenodeviants* as defined by Lerner[6], the abnormal morphology being due wholly or in part to level of homozygosity, *per se*. It should be mentioned that, among all exencephalic animals produced, there were approximately equal numbers of males and females.

Review of the literature indicates that the particular condition reported here has not been studied genetically, although it may possibly be known to some poultry breeders and geneticists. Exencephaly and related teratological conditions in the fowl were studied and reported by Dareste[2] in the last century. In a study of chick monsters in relation to embryonic mortality, Hutt and Greenwood[5] found 0.84 percent exencephaly among nearly 12,000 unhatched eggs. In their experience, exencephalic chicks rarely hatched, most of them dying during the third week of incubation; nor was there any good evidence of a genetic basis for the condition. Fowls of various crested breeds exhibit genetically-determined cerebral hernia, but not cranioschisis of the type reported in this paper. Instead the skull is enlarged, and, in mature birds, the roof of the cranium is vaulted and perforated with holes of various sizes[1,4]. Another case of inherited cerebral hernia is that reported by Marble *et al.*[7] Embryos homozygous for an autosomal recessive gene had missing and deformed mandibles and were unable to pip the shell, and in every instance affected embryos showed some degree of cerebral hernia.

From the standpoint of comparative genetics, it is interesting to note that conditions similar to that described here have been reported to occur, and to be inherited, in other species. A breed characteristic of Crested White ducks[1] is the large crest due to cranioschisis in the parieto-occipital region, resulting in encephalocele[4]. Crested ducks are said to be heterozygous for an incompletely dominant gene which is lethal in the homozygotes[4]. In mammals, exencephaly very similar to that in the present study, and presumed by its describers to be inherited, has been reported in both swine[3,8] and cattle[9].

Summary

An inherited exencephaly was discovered in S .C. White Leghorns of the University of Delaware strain. The condition was recessive, but the exact mode of inheritance was not apparent. Among exencephalic chicks, there was a wide range of expression of the trait, ranging from minute hydrencephalocele to massive herniation of the brain. No other gross abnormalities were apparent. Most exencephalic birds died in early life, but several were reared to maturity and used in breeding tests.

TABLE II. Incidence of exencephaly from matings of exencephalic fowls *inter se*, expressed as number exencephalic/total number classifiable

♂ # \ ♀ #	1956		1957			Sire totals
	6871	6872	422	423	425	
6873	18/63	1/61				19/124
6096			7/20	0/28	1/3	8/51
6099			3/26	0/41	5/15	8/82
Dam totals	18/63	1/61	10/46	0/69	6/18	Grand total 35/257

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CYTOGENETICS OF RUBUS

I. Reproductive Behavior of *R. procerus* Muell.

D. MARKARIAN AND H. P. OLMO*

THE Himalaya blackberry *Rubus procerus* Muell. is found in Asia, Europe, and the Pacific Northwest of America. Escaping from cultivation, it is an aggressive plant and has invaded large areas of northern California and the Pacific Northwest, along waterways and irrigation canals. It occurs only as a tetraploid ($2n = 28$), and its constancy in form suggests it is an apomict. The intent of the present work was a cytogenetic study of the reproductive mechanism of *R. procerus*, especially of its reputed apomictic behavior.

The origin of *R. procerus* is uncertain. According to Bailey[1], it was introduced in the early nineties by Luther Burbank from seed sent to him by an English traveler who collected it from the Himalaya mountains. Alfred Mitting[3], an American nurseryman, states the Himalaya blackberry was brought out of the Himalaya mountains by a British expedition and was first introduced into the United States by a Seattle firm in 1905. Bunyard[3] described the Himalaya blackberry as identical with Theodor Reimers, a variety found in Germany and which originated in 1889. Reimers selected the variety out of a progeny grown from a promising plant he saw in a neighbor's garden. Paul Dapp-Opplingen[4] describes this seed parent as *Rubus arenarius*, and Bunyard was convinced the description of this species fitted the variety Theodor Reimers. Rolph[17] describes the plant from which Reimers obtained the seed as *Rubus incarnatus* Moell. and the variety Theodor Reimers as a hybrid of this plant.

The historical evidence indicates *R. procerus* did not originate in America or the Himalaya mountains and probably originated in Germany. The variety Theodor Reimers is apparently identical with *R. procerus* and if the origin of the former can be established, then *R. procerus* will be identified.

Darrow[7] reported genetic variation in natural populations of *R. procerus*. However, seedlings he grew from selfed plants were all identical, indicating apomixis. Darrow and Waldo[8], working with *R. procerus*, used 11 different pollen parents and obtained a total of 320 false seedlings and 183 true seedlings, 64 and 36 percent respectively. Crane[6] reported only sterile seed when *R. procerus* was selfed. However, seed he collected in nature gave plants reported identical to the parent. Kerr[12] indicated approximately 24 percent of Himalaya drupelets from flowers pollinated with Evergreen pollen (*R. laciniatus*) possess normal appearing seed. Germination trials were not made; consequently, fertility and apomixis could not be reported.

Although these earlier reports leave some question regarding self fertility, it is apparent that selfed progenies, whether from spontaneous plants or from cultivated material, are entirely psuedogamic and crosses with other species also give a large proportion of psuedogamous plants.

Materials and Methods

Four clones of *R. procerus* were studied, one growing naturally at Natomas, California, and three domestic ones at Davis, California. All crosses were enclosed in cheese cloth bags to prevent pollen contamination. Drupes were harvested at maturity, and nutlets

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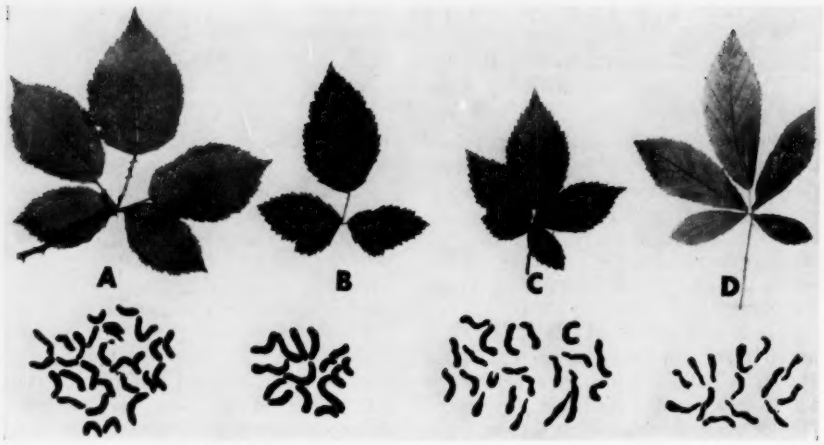

MITOTIC MEATPHASE PLATE AND TYPICAL LEAF
Figure 10

A—R. pocerus; B—haploid *R. procerus; C*—hybrid of *R. procerus* × *R. louisianus; D—R. louisianus.*

were extracted with a Waring blender. First year germination could only be obtained by treating the nutlets with sulphuric acid[11]. Pre-blossom embryo sac studies were made from the intact drupe; 248 drupes of various sizes were studied. They were fixed in Randolph's modified Navashin fluid[16] for 24 hours and dehydrated in the butyl series. The larger drupes were treated in 25 percent HFl acid for five to 30 minutes in order to soften the tissues. After 30 minutes' washing they were dehydrated in dioxan[2]. Embedding was in tissue mat and sections were made from six to 12 microns. All were stained by Stockwell's schedule[18].

Post pollination ovules were best studied if they were dissected from the pistil before fixation; 208 ovules were dissected and studied. They were dehydrated in the butyl series and staining was in iron-haematoxylin[17]. The schedule was modified by diluting the mordant to 0.2 percent, and 1.0 percent aqueous safranin was used as a progressive strain.

Root tip chromosomes were studied by the paraffine technique. Fixation was in Randolph's fluid, followed by dehydration in the butyl series. They were cross sectioned at 12 microns and stained progressively in one percent aqueous crystal violet. Chromosome drawings were made from photomicrographs.

Experimental Results and Discussion

Self Fertility

Rubus procerus is a relatively infertile species (Table I). Of the few plants obtained from the germination trials, a high percentage were haploids, thus further reducing normal reproductive ability. Crane[6] reported the species sterile and actually, had not large num-

TABLE I. Seed germination of self- and open-pollinated *R. procerus*

Clone	Location	Type of pollination	No. of seeds planted	No. of seeds germinated	Percent germination	No. of haploids	Percent haploids
B	Davis, California (greenhouse)	self	534	38	7.12	5	13.1
A	Folsom, California	open	10,140	64	0.63	5	11.4
B	Davis, California	open	6,610	33	0.48	2	6.3
B	Davis, California	self	10,230	8	0.08	2	25.0

bers of seed been used in obtaining the results of Table I, the present findings may well have supported this earlier report. Clone B, grown under greenhouse conditions, produced a considerably higher percentage of viable seed, and it may be presumed that field conditions less extreme than that found at Davis or Folsom, California, might yield a higher percentage of viable seed.

The observed and reported low seed fertility of *R. procerus* may be the result of a single or several interrelated phenomena. The species is probably of hybrid origin as interspecific hybrids in *Rubus* are often found under natural conditions. There is evidence that it may be a hybrid of rather closely related species: Mallah[14] found a range of 0-3 and an average of 1.34 quadrivalents in an examination of 115 pollen-mother-cells. The observance of quadrivalents may be used as evidence for autotetraploid origin. The haploid plants observed in the present study were extremely uniform and support this latter argument.

Hybridization

The almost complete failure of hybridization (Table II) is no doubt at least partly a result of the inherent poor germinability of *R. procerus* seed. The two crosses utilizing "Lawton" and "Snyder" were a duplication of species used by Darrow and Waldo[8] which they were able to successfully cross with *R. procerus*. This discrepancy plus the fact that the only successful cross in the present study (Table I) was made in the greenhouse indicates the large role of environmental effects on fertility. The possibility also exists that the clones used by Darrow and Waldo represented a genetically more fertile type.

Figure 10*A-D* illustrates mitotic metaphase drawings and leaf morphology of *R. procerus* (Figure 10*A*), haploid *R. procerus* (Figure 10*B*), *R. procerus* × *R. louisianus* hybrid (Figure 10*C*), and *R. louisianus* (Figure 10*D*). Leaves of the hybrid plant (Figure 10*C*) appear intermediate between the parents and the expected number of chromosomes (21) are present.

Apomixis

The genus *Rubus* has been demonstrated to be represented by a number of European[10] and North American[8,9,15] apomictic (pseudogamic) forms. Darrow and Waldo[8] and Crane[6] indicated genetic segregation did not occur in selfed progenies of *R. procerus*.

In the present study both the self- and open-pollinated progenies of *R. procerus* were considered as selfs since the clones were well isolated and actually flowered at a time when other species were still in bud. All of the 143 seedlings of Table I were space planted and held in the field for two years. The second year primocanes of these plants afforded ample evidence of genetic segregation. Differences in hairiness, leaf shape, in addition to differences in plant habit and leaf color were noted. The accepted technique for detecting apomixis is hybridization of the suspected plant (female parent) with a species or variety sufficiently different to permit detection of true hybrids. Obviously then, selfed progenies would be (and were in this study) a poor source of evidence for apomixis even where qualitative differences did not exist in the progenies. Quantitative differences of a very subtle nature could be present in a relatively homozygous species, and the indeterminant growth habit of blackberries would render analysis very difficult. The appearance of segregation in selfed progenies in the present study is not in accord with the accepted apomictic behavior in this variety. In addition, it would appear that if the species tended toward apomixis the one successful

TABLE II. Results of crosses performed to detect apomixis in *R. procerus*

Female parent	Male parent	Somatic chromosome number	Flowers pollinated	No. of berries set	Total no. of seed	True seedlings	False seedlings
R. procerus	*R. idaeus* var. 'Chief'	14	41	0	0	0	0
R. procerus	*R. idaeus* 'Latham'	14	38	0	0	0	0
R. procerus	*R. louisianus* 'Crystal'	14	125	5	7	0	0
R. procerus	*R. philadelphicus* 'Lawton'	28	182	130	1201	0(8)*	0(9)*
R. procerus	*R.* ? 'Mersereau'	28	158	3	4	0	0
R. procerus	*R. rosa* 'Eldorado'	28	111	4	7	0	0
R. procerus	*R. rosa* 'Snyder'	28	47	2	2	0(7)*	0(27)*
R. procerus	Lawton Type 5105	28	22	6	39	0	0
R. procerus	Lawton Type 5102	28	22	12	101	0	0
R. procerus	Lawton Type 5103	28	23	12	39	0	0
R. procerus	*R. ursinus* 5104	56	19	1	1	0	0
R. procerus	*R.* 'Macatawa'	28	24	12	78	0	0
R. procerus	*R. louisianus* 'Crystal'	14	many	9	60	1	0

*Results from Darrow and Waldo[8]

greenhouse cross (Table II) should have been accompanied by false hybrids.

Development of the Embryo Sac

Ovule development in *R. procerus* follows the pattern described by Christen[5] in eight *Rubus* species and Pratt and Einset[15] in seven species. Essentially in all, the carpel contains two anatropous, crassinucleate ovules, one attached above the other—the upper one aborting during early development. This form of development no doubt typifies the genus.

Archesporial development, however, is not so easily categorized as might be expected from the numerous reports of pseudogamy within the genus[9,10]. Christen[5] presents a classification based on archesporial development as interpreted from the species he studied. At one extreme he places *R. caesius* in which the entire archesporium is occupied by sporogenous cells (secondary archesporial cells), all of which can produce megaspore mother cells. At the other extreme he places such species as *R. vestitus* in which the entire archesporium may appear as undifferentiated somatic cells. Intermediate to these extremes are two classes that represent a gradient of the number of secondary archesporial cells produced, and their ability to produce megaspore mother cells.

The position of *R. procerus* in this scheme is complicated by a number of relevant facts. The very low seed germination from naturally growing plants (Table I) indicates less than one percent of the ovules observed cytologically would give the correct interpretation of embryo sac development (assuming the observed sterility to be a function of the female gametophyte). Evidence of megaspore mother cell formation (Figure 11*A*) and subsequent meiosis (presence of three degenerating megaspores, Figure 11*B*), was rare. Where it was observed, however, the evidence indicates that only a single cell was involved (primary archespore) and this being at such an extremely early stage of ovule development that it was impossible to establish cell differences that would permit delineation of sporogenous and somatic tissue. Kerr[12] also reports embryo sac origin from a single archesporial cell in Eldorado and BB-1 and BB-2 varieties. *R. procerus* would seem to best fit into the category of *R. vestitus* in Christen's scheme since at the very early stages of ovule development all areas of tissue (including the epidermis) appear similar. Megasporogenesis occurred at an early bud stage (2-3 mm diameter); studies of the later stages in larger buds gave no evidence that the nucellar tissue had generative ability.

Figure 11*A-D* illustrates the only form of embryo sac development found in this variety and it is of the commonly designated "normal type"[13]. The antipodal cells stained heavily (Figure 11*D*) and the egg apparatus appeared normally situated at the micropylar end of the embryo sac. The two centrally located polar nuclei were never fused prior to fertilization.

Pollination and Embryo Development

Pollen germination was good following self-pollination. The pollen tube enters through the micropyle directly into the area of the egg apparatus. Figure 10 illustrates the enlarged end of the tube prior to eruption. The pollen tube enters the embryo sac within 24 hours after pollination under greenhouse conditions (70° F.). The male nuclei were rarely seen, and actual fusion was not found. Two factors probably contribute to this failure: Fusion is no doubt a rapid process and the discharge from the pollen tube results in a very densely staining protoplasmic material that masks the egg apparatus and usually the polar nuclei. Maheshwari[13] reports this condition to be true in most higher plants. At the time of pollination the polar nuclei are often found quite close to the egg. Synergids are rarely identifiable as intact cells at the time the pollen tube enters the embryo sac.

The early embryo is surrounded with abundant endosperm consisting of large free nuclei suspended in densely staining cytoplasm (Figure 11*G*). The endosperm decreases in amount as the embryo develops, and at ten days is nearly depleted.

The Development of the Embryo and Drupe

In *R. procerus* each drupe represents an aggregate of approximately 60 drupelets. However, only about 50 percent of these reach a mature edible state. The mature drupelets are quite variable in size. Kerr[12] indicates similar results in tabulated form. He observed further that "until six days after pollination there was an intergraduation between three types of drupelets—undeveloped, poorly developed, and well developed." Similar observations were made in the present study. In collection of drupelets for study of embryo development, pollination date was recorded and the druplets were selected on the basis of development. From the well developed drupelets at three to four days after pollination, normally developing embryos were found. However, the poorly developed drupelets never formed embryos. This observation offers evidence that considerable drupelet sterility may be due to embryo sac failure and failures at megasporogenesis. The complete somatic appearance of the nucellus of older buds mentioned earlier and described by Pratt and Einset[15] may account for most of the drupelet sterility. However, an undetermined percent must be the result of situations illustrated in Figure 11*H* and *I*. Figure 11*I* is probably an egg cell after two divisions (four cells); no endosperm exists and possibly fertilization was not effected. The four cells could well be haploid. Figure 11*H* offers the only evidence for the formation of haploids. Cell (E) is late in development compared to the embryo of Figure 11*G*. The remains of the pollen tube

LONGISECTIONS OF *R. PROCERUS* OVULES

Figure 11

A—Megaspore mother cell; *B*—functional megaspore and degenerating megaspores (DM); *C*—two celled E.S.; *D*—four celled E.S.; *E*—eight celled, complete E.S.; *F*—two pollen tube ends having entered micropylar end of E.S.; *G*—developing embryo (EM) surrounded by endosperm; *H*—unidentified lagging cell (E) may be either male or female gamete since pollen tub (PT) has already entered, endosperm appears normal; *I*—four celled embryo with no surrounding endosperm.

(PT) and the normal appearing endosperm indicates at least that the polar nuclei were fertilized. The undetermined cell (E) could be a fertilized egg cell; however, since it has lagged in development it is more likely either the haploid egg cell or the male gamete which could eventually form a haploid embryo.

The present evidence of a completely sexual form of embryo sac development in *R. procerus* (a previously reported apomictic species) finds a striking parallel in Christen's report of *R. caesius*. This species has been described by Lidforss[10] as the only completely apomictic *Rubus* species. Christen[5] reports counts of 12-13 chromosomes at anaphase ($2n = 28$) of the third division in the embryo sac indicating meiosis occurs, and further that normal embryo sac development was all that he was able to find. Unfortunately Christen did not test his forms of *R. caesius* for apomixis. His conclusion was that his material may have been genetically different from the Swedish forms used by Lidforss.

Summary

A high degree of sterility was found in *R. procerus* under central California conditions.

The evidence supports three causal factors for this sterility: (1) the appearance of a large percentage of maternal haploids in selfed progenies indicates a failure of fertilization, (2) the absence of embryo sacs in many of the drupelets indicates failure of the megaspore mother cell, (3) both (1) and (2) are a measure of the efficiency of meiosis, therefore, the sterility observed may be attributed to failures at meiosis.

Environmental factors may markedly influence self-fertility in this species as evidenced by a considerable increase in germination percentage of seed grown under greenhouse conditions.

The evidence presented does not support the earlier reports of apomixis in this species. This discrepancy may be due to genetic variation or may simply be a question of differential response under variable environment. Assuming apomixis on the basis of maternal resemblance of the young seedlings is not warranted, especially in autoploids like *Rubus procerus*.

Since genetic segregation has been shown to occur on selfing, it is likely that the wide discrepancies in fertility and apomixis previously reported can be best explained on this basis.

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A LETHAL MUTATION IN DORKING FOWL

WALTER LANDAUER*

IN a pen mating of Silver Gray Dorking fowl two hens were observed in 1955 whose fertile eggs had an abnormally high embryo mortality during the second week of incubation (Pen 34, Table I). A progeny was, therefore, raised for further observation (Pen 7—1956). We also added a few White Leghorn pullets to Pen 34—1955 in order to test the resulting F_1-birds for the presence of a lethal mutation (Pen 31—1956).

Our observations in 1956 clearly established an excessive embryo mortality in certain individual matings. The major part of this mortality occurred during the eighth day of incubation, but some of it took place during the early part of the ninth day. By opening eggs prior to and following the peak of mortality it could be ascertained that embryos which are destined to die have certain morphological features in common (Figure 12), and that the number of embryos which are identifiable in this manner does not deviate significantly from that expected in the presence of a lethal mutation (see below). The lethal embryos can readily be identified at the age of seven days and probably in still earlier stages.

NORMAL AND MUTANT DORKING FOWL

Figure 12

Normal embryo left and short-neck embryo right after eight and one-half days of incubation.

The most striking gross abnormality of these lethal embryos is the shortness of the neck. Beak development is always abnormal to some extent. Growth of the mandible is retarded; the lower beak is short and often in an abnormal position with its tip pointing in a vendrad direction; there tends to be a gap, often a large one, between lower and upper beak. The upper beak is short and frequently curves downward. The tail is usually very short. The extremities may be shortened relative to body size, but this is not a regular feature. Body weight tends to be 15 to 25 percent below normal. Methylene blue staining of whole embryos shows a retardation of cartilage formation in the mandible and in the cervical and tail vertebrae, but the number of vertebrae is normal and they are not grossly malformed. This was confirmed by histological preparations. No clear differences were found in other parts of the skeleton. As the only non-skeletal abnormality we observed that the eyes of the lethal embryos tend to be somewhat more heavily pigmented than those of their normal sibs. Since shortness of the neck is by far the most conspicuous feature of these embryos the mutation will for convenience be referred to as the "short-neck" lethal.

During 1957 and 1958 we conducted further breeding tests with descendants of the Dorking stock (Pen 8—1957 and 7—1958) and with F_2 and F_3-birds from the Leghorn $\times$ Dorking cross (Pens 27, 28 and 29—1957; 27, 29 and 30—1958). The results of all matings are reproduced in Table I. Individual matings which yielded less than 16 fertile eggs were omitted from the tabulations. Altogether we have records of 96 hens which

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appeared to be heterozygous for the short-neck lethal condition. In matings with heterozygous cocks these 96 hens produced 3458 fertile eggs; the mortality during the first six days amounted to 4.5 percent, from seven to 13 days 24.2 percent of the embryos died, and hatched chicks emerged from 58.8 percent of the eggs. In contrast, 58 hens which obviously did not carry the lethal genes produced 2049 fertile eggs with a mortality of 3.7 percent during the first six days and of 2.6 percent between seven and 13 days of incubation; the hatch amounted to 75.6 percent. In the *inter se* matings of heterozygous birds 3301 embryos survived the sixth day of development and 25.3 percent of these died between seven and 13 days; in the matings of non-heterozygous hens to heterozygous cocks 1973 embryos survived the sixth day and of these only 2.7 percent died during the second week of incubation. After the proper correction for mortality in the control groups, a χ^2 calculation shows that the seven to 13 day mortality of embryos from the presumed heterozygous parents does not deviate significantly from that expected in the presence of a recessive lethal mutation.

In some of our matings during 1957 and 1958 the same heterozygous hens were first bred to a non-heterozygous and afterwards to a heterozygous cock. In addition, we had data for *inter se* matings of non-heterozygous birds which were the offspring of heterozygous parents. The relevant data are shown in Table II. The data for the same heterozygous females mated first to non-heterozygous males and afterwards to heterozygous ones are very similar to those shown in Table I and which have already been discussed. It is of interest, however, to make a comparison of embryo mortality in the first and last group, i.e. the matings of heterozygous hens to non-heterozygous cocks versus matings between extracted non-heterozygous birds. It can be seen that in the first group embryo mortality was higher than in the last one, especially between seven and 13 days, and this difference is highly significant ($\chi^2 = 9.12$, $P < .01$). Whether this means that embryos which are heterozygous for the lethal mutation

TABLE I. Incubation records of Silver Gray Dorking fowl and of outcrosses of Dorkings to White Leghorns, according to whether or not the mated females were heterozygous for the short-neck lethal. The cocks in all matings were heterozygous for the lethal

Pen and year	Genotype of ♀♀	Number of ♀♀	Fertile eggs	Mortality during incubation days % 1-6	7-13	14-22	Hatched %	Mortality during 7-13 day period among survivors of 6th day in %
34—1955	Het.	2	53	0	32.1	45.3	22.6	32.1
	Non-het.	6	225	7.1	4.4	42.7	45.8	4.8
7—1956	Het.	6	183	3.8	26.8	4.4	65.0	27.8
	Non-het.	2	66	3.0	4.5	6.1	86.4	4.7
31—1956	Het.	10	374	2.1	23.5	3.4	70.9	24.2
	Non-het.	12	395	2.0	1.3	2.8	93.9	1.3
8—1957	Het.	13	370	6.8	26.5	14.9	51.9	28.4
	Non-het.	2	44	4.5	4.5	31.8	59.1	4.8
27—1957	Het.	9	425	3.5	21.4	13.6	61.4	22.2
	Non-het.	6	191	2.1	4.7	8.4	84.8	4.8
28—1957	Het.	4	114	2.6	31.6	21.9	43.9	32.4
	Non-het.	5	133	6.8	3.0	33.8	56.4	3.2
29—1957	Het.	12	518	1.2	23.7	13.9	61.2	24.0
	Non-het.	5	172	3.5	3.5	21.5	71.5	3.6
7—1958	Het.	14	444	8.1	25.0	13.1	53.8	27.2
	Non-het.	3	118	2.5	0	11.0	86.4	0
27—1958	Het.	10	366	6.6	22.4	10.1	60.9	24.0
	Non-het.	5	244	2.0	0.4	8.2	89.3	0.4
29—1958	Het.	8	304	3.0	20.1	8.9	68.1	20.7
	Non-het.	6	216	6.8	2.8	15.3	75.9	3.0
30—1958	Het.	8	307	7.8	26.1	17.6	48.5	28.3
	Non-het.	6	245	3.3	3.3	9.4	84.1	3.4

and descended from heterozygous mothers are at a disadvantage compared to those free of it or compared to heterozygotes produced by heterozygous fathers must be tested in future experiments.

Among 92 daughters of heterozygous parents, for which adequate tests are available, 60 carried the lethal and 32 did not. This agrees very closely with the 2:1 expectation for such matings. Among 51 daughters of non-heterozygous mothers and of heterozygous fathers 30 were heterozygous and 21 were not, a non-significant deviation from the expected equality of classes.

Discussion

It is clearly established by the foregoing data that a single recessive gene substitution is responsible for the excessive embryonic mortality that occurred in our matings of Dorking fowl and in the descendants of outcrosses between this Dorking stock and White Leghorn fowl. The gross effects of the mutation are on growth of definite skeletal parts, but the modifications of eye pigmentation indicate that more subtle developmental processes (migration of neural crest cells?) are also involved, and the point of interference with vital processes remains to be determined. The short-neck mutation is somewhat unusual among lethal mutations of fowl in the time at which it causes death of the homozygotes; in the majority of lethal mutants of fowl death occurs much later or considerably earlier than is true for the short-neck lethal.

There are superficial points of similarity between the short-neck mutant, described here, and the crooked-neck dwarf and short-spine lethal mutations of chickens and turkeys (Asmundson[1,2]; Asmundson and Pun[3]; Pun[4]). Although the details of malformation differ between these mutants, one may be justified in concluding that growth and differentiation of the cervical spine are especially prone to mutational disturbance.

Summary

A recessive mutation has been found in Dorking fowl which causes death of the embryos during the eighth or the beginning of the ninth day of incubation. The body size of such embryos is somewhat reduced, the neck is very short, the beak is abnormal, the tail is often short and the eyes tend to be more heavily pigmented than those of their normal sibs.

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TABLE II. Data of incubation for matings in which hens heterozygous for the short-neck lethal were first mated to non-heterozygous and later to heterozygous cocks, and also of *inter se* matings of extracted non-heterozygous birds

Type of mating	Number ♀♀	Fertile eggs	Mortality during incubation days % 1—6	7—13	14—22	Hatched %
Heterozygous ♀♀ × non-heterozygous ♂	22	354	2.0	9.0	12.7	76.3
Heterozygous *inter se* (same ♀♀ as above)	22	787	6.5	25.2	14.7	53.6
Extracted non-heterozygous *inter se*	14	306	2.3	2.0	10.1	85.6

A NEW DOMINANT T-ALLELE IN THE HOUSE MOUSE

MARY F. LYON*

THE *T*-locus of the house mouse (*Mus musculus*) is well known for the unusual properties of the numerous recessive *t*-alleles[3]. Mutations to dominant mutant alleles at this locus have been detected at least twice, and possibly three times, but these dominant alleles have been indistinguishable from each other[1,2]. Heterozygotes show the shortened tails which give the name brachyury to the allele, and homozygotes die at about 10 days' gestation with reduction of the posterior part of the body, absence of the hind limbs and tail, and a reduced allantois. The present paper describes a new short-tail mutation and presents evidence that the new gene, named T^h, is allelic with the *T*-series but is different from the already known dominant mutant allele, *T*.

Origin and Genetics of the Mutant

The original mutant animal, a female with a tail about half normal length, was found among the offspring in the control series of a mutagenesis experiment.

When outcrossed to a normal male this female produced 36 offspring, 16 short-tailed, two tailless and 18 normal; her short-tailed offspring when mated to wild-type produced 95 short-tailed and 85 normal young (Table I), suggesting that the short-tailed condition was due to a dominant mutant gene. The new gene was next tested for allelism with two known dominant genes affecting the tail, *Sd* and *T*. Allelism with *Sd* was tested by looking for typical *SdSd* homozygotes in crosses of *Sd*+ females with males heterozygous for the new gene; no *SdSd* animals were found among 21 offspring of such crosses.

Allelism with *T* was tested in three ways. First, the chromosomal position of the new gene was determined by linkage tests with tufted, *tf*, which shows about eight percent recombination with T^4; the new gene also showed close linkage with *tf*. Second, the new gene was tested for interaction with t^{13}; this test was made because the mutants fused, *Fu*, and kinky, Fu^{ki}, which also affect the tail, lie very close to *T* in linkage group IX but are not allelic with it; they differ from *T* in that they do not interact with the *t*-alleles to give a tailless heterozygote. Third, the characteristic *TT* embryos were looked for in crosses of *T*+ females with males heterozygous for the new mutant.

Results of Tests of Allelism with *T*

The results of the first two tests, taken together, indicate allelism of the new gene with *T*; it was therefore given the symbol T^h. In T^h+/+*tf* heterozygotes T^h shows 4.9±2.4 percent recombination with *tf*, which is consistent with the eight percent found in *T*+/+*tf* heterozygotes (Table II). When T^h+ males were crossed with $+t^{13}$, nine out of 58 offspring were tailless and these tailless

TABLE I. Single factor segregation of T^h

Parents ♀	Parents ♂	Offspring: Short tailed	Tailless	Normal	Total
T^h+ (original mutant)	++	16	2	18	36
T^h+	++	33	..	20	53
++	T^h+	62	..	65	127
$+t^{13}$	T^h+	17	9	32	58

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offspring gave short-tailed and normal young when crossed to wild-type. This is consistent with the interpretation that $T^h t^{13}$ animals are tailless.

The examination of embryos resulting from crosses of $T+$ females with T^h+ males, however, yielded equivocal results. Of 73 embryos examined at 10 to 11 days' gestation eight showed the characteristic defect of TT embryos, with hind legs and tail lacking (Table III). This is significantly less than the 25 percent abnormal that would be expected if TT^h embryos resemble TT and at present no explanation is offered for this discrepancy. Perhaps some TT^h embryos die earlier than TT; or conceivably some might survive until later and appear normal at 10 days, but this point has not yet been investigated.

Evidence that T^h Differs from T

To identify T^hT^h homozygotes the embryos of T^h+ females crossed with T^h+ males were studied; the results are shown in Table III. Seven litters totalling 53 embryos were examined at 10 to 11 days' gestation; no embryos showed the typical defects of TT but 14 had died at earlier stages and were represented by "small moles." Another series of similar females were opened at eight days' gestation. Roughly one quarter of these embryos showed a characteristic abnormality; there was a small embryo sac, about half normal size, containing only a small cone of tissue attached to the mesometrial wall of the sac. Out of 115 eight-day embryos 25 were of this type; this is in good agreement with the 25 percent expected if these were the T^hT^h embryos. It is therefore thought that T^hT^h embryos die at an earlier stage than TT and that T^h is a different allele from T.

On the other hand the heterozygotes of T^h with $+$ and with t^{13} phenotypically resemble $T+$ and Tt^{13}; if there are any differences comparison on carefully matched backgrounds would be required to detect them and such a comparison has not been made. T^h also resembles T in showing normal segregation ratios, whereas some *t*-alleles segregate abnormally when carried in males.

TABLE II. Linkage tests between T^h and *tf*

Parents ♀	Parents ♂	Offspring T^h+	Offspring T^htf	Offspring $++$	Offspring $+tf$	Total	Recombination %	Recombination S.E.
$T^h+/+tf$	$+tf/+tf$	29	1	3	36	69	4.94	2.41
$+tf/+tf$	$T^h+/+tf$	5			7	12		

							Recombination %	Recombination Upper fiducial limit
$T^h+/t^{13}tf$	$+tf/+tf$	31			43	74	0	3.1%
$+tf/+tf$	$T^h+/t^{13}tf$	20			25	45	0	5.1%

TABLE III. Counts of embryos in T^h crosses

Parents ♀	Parents ♂	No. of litters	Age in days	Embryos Normal	Embryos Small moles	Embryos Typical *TT*	Embryos Others	Total
$T+$	T^h+	7	10	61	3	8	1	73
T^h+	T^h+	7	10-11	36	14		3	53
					Small embryo sac with cone of tissue	No embryo sac		
T^h+	T^h+	3	9	11	3	8	6	28
		14	8	76	25	7	7	115

Further work is needed, however, to decide whether T^h differs from T in its linkage relationships with tufted. Some t-alleles suppress crossing-over between T and nearby loci. Lyon and Phillips[5] found that t^6 suppresses crossing-over between T and tf almost completely, whereas among Tt^{13} heterozygotes crossing-over is normal in females and reduced but not abolished in males. Table II shows that in $T^h+/t^{13}tf$ heterogygotes no recombination has so far been observed. Further data are needed to determine whether any recombination does in fact occur in T^ht^{13} animals and, if so, with what frequency; but the data are already sufficient to show that in female heterozygotes the upper fiducial limit of the recombination at the five percent probability level is well below the recombination found in Tt^{13} females. T^h thus seems to differ from T in its linkage relations when t^{13} is present. The recombination between T^h and tf *in* $T^h+/+tf$ heterozygotes also appears to be lower than that found in $T+/+tf$ heterozygotes, but the difference is so far not statistically significant.

Discussion

It is interesting to have another dominant allele at this locus. If the apparently different linkage relations of T^h are substantiated this will be especially interesting in view of the suggestion that the properties of t-alleles are due to an abnormal chromosome region, as it will suggest that the dominant alleles also are not "point" alleles but involve an appreciable length of chromosome. The suggestion that the t-alleles involve an abnormal region of chromosome was given support by the finding[5] that "mutation" of t-alleles was accompanied by crossing-over between T and tf, suggesting that the mutation was in fact not mutation but crossing-over. In the case of T^h it is not known whether the mutation that gave rise to it was accompanied by crossing-over as neither parent of the mutant animal carried any marker genes in this linkage group.

Summary

A gene which arose by spontaneous mutation and which when heterozygous results in shortening of the tail was found to be an allele at the T-locus and has been given the symbol T^h. It differs from the already known dominant allele T in that homozygotes die at an earlier stage of gestation, being already highly abnormal at eight days.

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A HYDROCEPHALIC LETHAL IN HEREFORD CATTLE

R. L. Blackwell, J. H. Knox and Estel H. Cobb*

TWO ABNORMAL CALVES
Figure 13

Two abnormal, new-born calves (*A*) and (*B*) and close-up views of their heads (*C*) and (*D*). The bulging forehead is clearly apparent in these calves. The calf in photo (*A*) lived for a short time while the one in photo (*B*) was dead at birth.

A LETHAL which appeared in the experimental herd of Hereford cattle at the New Mexico Agricultural Experiment Station has been studied to determine its mode of inheritance and to obtain descriptive data. The lethal reported here is characterized by internal hydrocephalus, but certain other abnormalities associated with the lethal condition have not, to the authors' knowledge, been reported in the literature. Cole and Moore[2] reported a hydrocephalic lethal in Holstein cattle along with asymmetry of skull and face, malformation of humeri and femurs, and a nervous condition referred to as "jumpy." They suggested three independent recessive genes as the genetic explanation of these abnormalities. Johnson *et al.*[4] and Pahnish *et al.*[5] report hydrocephalus in Hereford dwarfs, but the hydrocephalic condition apparently was not always associated with this type of dwarfism. Hydrocephalus in the Marche breed of cattle reported by Giannotti[3] was presumed to be due to a single recessive gene. Clark[1] presents evidence that two types of hydrocephalus in mice

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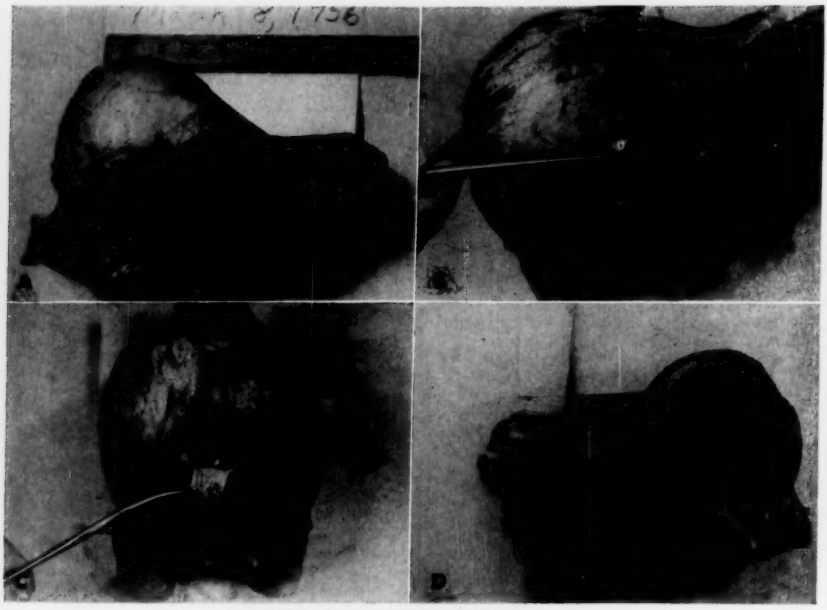


EXTREME HYDROCEPHALUS

Figure 14

Extreme case of hypdrocephalus showing cranial bulge (*A*), malformed supraorbital foramen (*B*), incomplete ossification of the frontal bones (*C*), and extreme hydrocephalus (*D*). Note the open spheno-occipital juncture (indicated by the ditsinct line of epiphyseal cartilage) and small cerebellum.

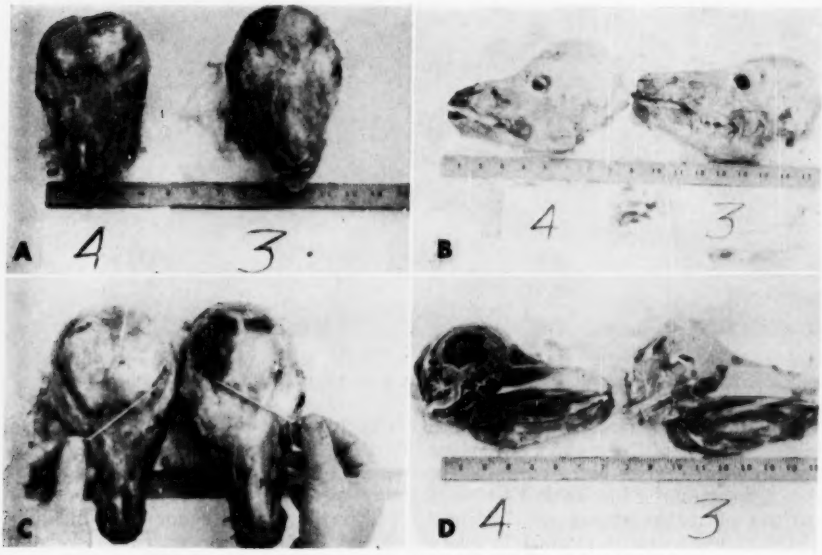
depended upon different genes for expression. Personal communications with researchers working with beef cattle indicate that losses from hydrocephalus may be of some importance economically.

Description

The affected calves have been dead at birth or have died shortly thereafter. In cases where such calves were observed alive, none were able to stand or make direct motor movements. Small body size and a bulging forehead have characterized all affected calves (Figure 13). The teeth have generally been incompletely developed (Figure 13*D*).

The only anatomical abnormalities observed upon autopsy have been those of the skull and the brain. Brain examinations performed on sagittal sections of the frozen head revealed marked internal hydrocephalus. In the more extreme cases incomplete ossification of the skull was encountered (Figure 14*C*). The cerebella of affected calves were smaller than those of normal calves (Figure 15*D*).

The supraorbital foramina of affected calves were almost completely occluded and the supraorbital grooves were shallow. According to Sisson and Grossman[8] the supraorbital foramen is often double in the bovine. The foramina observed in these abnormal calves frequently have been several in number consisting of a small foramen (the major one) accompanied by several much smaller ones. The small ones have been rather widely dispersed about the area of the supraorbital groove. (See Figures 14*B* and 15*C*.) Normally the major foramen in the newborn calf is about two to three millimeters in diameter and the groove is palpable in the live calf. In the abnormal



HEADS OF NORMAL AND HYDROCEPHALIC CALVES

Figure 15

Comparison of heads from a normal calf (no. 3) and a hydrocephalic calf (no. 4). Front and side view.

calves autopsied, malformation of the supraorbital foramen has accompanied the hydrocephalic condition without exception. Normal calves in this herd that died at birth or shortly thereafter for various reasons were examined. None were found to be hydrocephalic, and their supraorbital foramina were always normal. Figure 15 illustrates the anatomical differences between a normal and an abnormal head.

This hydrocephalic condition is considered to be associated with and possibly the cause of the malformed supraorbital foramen. The dome shaped skull, the partial occlusion of the supraorbital foramen, the flattening of the supraorbital groove are probably caused by pressure exerted by excessive cerebrospinal fluid in early embryonic development. Since all forms of hydrocephalus apparently do not affect this foramen, this abnormality indicates a difference in the cause of the hydrocephalic condition.

TABLE I. Means and standard deviations (S.D.) of body measurements for normal and hydrocephalic calves

Body Measurement	Normal*			Hydrocephalic		
	No.	Mean	S.D.	No.	Mean	S.D.
Birth weight, pounds	142	68.2	10.8	9	40.2	6.9
Wither height, inches	48	25.5	1.4	9	22.5	1.7
Body length†, inches	48	23.5	1.5	9	19.6	1.2
Chest circumference, inches	48	26.4	2.0	9	22.1	1.3
Cannon bone length‡, inches	47	5.6	0.4	10	5.0	0.4

*Includes some calves that died at birth because of dystocia and some that died either at birth or shortly thereafter for unknown reasons.

†Body length was measured from the spinous process of the first thoracic vertebra to the pin bone (tuber ischii).

‡Cannon bone length measurements were made on the intact animal, therefore may not be an exact measure of metacarpal length.

AMNIONIC FLUID
Figure 16
Excessive volume of amnionic fluid accompanied the birth of a few hydrocephalic calves.

Certain body measurements were taken on some of these abnormal calves and on normal calves in the herd. These data are summarized in Table I. A comparison of means shows the hydrocephalic calves to be subnormal in every measurement. All differences were statistically significant at the one percent level of probability. The mean values for birth weight, wither height, body length, chest circumference, and cannon bone length of the abnormal calves were 58.9, 88.2, 83.4, 83.7, and 89.2 percent of the respective mean values of normal calves.

The ratios of birth weight, body length, chest circumference, and cannon length to wither height were computed for the normal and abnormal calves using the mean values from Table I. These values are presented in Table II. The ratios involving body length, chest circumference and cannon length were essentially the same for the hydrocephalic and the normal calves. The ratio of birth weight to wither height, however, was 1.79 for the hydrocephalic calves compared to 2.67 for normal calves. The difference between these two ratios is exaggerated somewhat because most of the normal calves were weighed alive, usually after they had nursed, while the abnormal calves were dead when weights were obtained and some dehydration may have occurred.

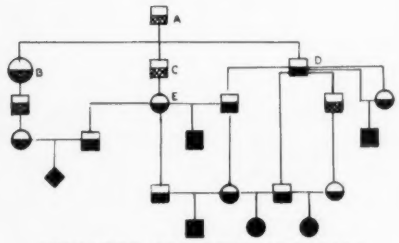
One apparent effect of this lethal is that of reducing prenatal growth. Also, it appears that the lethal process affects birth weight more adversely than skeletal size. Body length and chest circumference were more seriously affected than wither height and cannon length, indicating that the appendicular skeleton was affected less than the axial skeleton.

The length of gestation for these abnormal calves has been poorly established since the majority were born in the grade herd where breeding dates were not obtained. Five lethals born in the purebred herd had gestation lengths as follows: 281, 273, 279, 281, and 241 days. One abnormal calf in the grade herd was dropped 275 days after the beginning of the breeding season during which conception occurred. Schwarz[7] found the average gestation length in this purebred herd to be 285.6 days. Although the appearance of the teeth and the low birth weights indicated short gestations, most of these abnormal calves were only a few days premature.

The 245-day bovine fetus is reported by Winters *et al.*[9] to weigh approximately 59 pounds and have a chest cir-

TABLE II. Ratios of body measurements to wither height for normal and hydrocephalic calves

Ratio	Normal	Hydrocephalic
Birth weight/Wither height	2.67	1.79
Body length/Wither height	0.92	0.87
Chest circumference/Wither height	1.04	0.98
Canon length/Wither height	0.22	0.22



PEDIGREE OF HYDROCEPHALIC CALVES

Figure 17

Pedigree showing introduction of lethal gene through sire A and typical matings that produced hydrocephalic calves. Old line mates omitted for simplicity. Squares represent males; circles, females; diamond, sex unknown. Solid shaded symbols represent hydrocephalic calves; solid half-shaded symbols represent animals incriminated as carriers through their descendants. The coefficients of relationship between 19 lethals and bulls A and D averaged 21 percent and 38 percent, respectively. The coefficients of relationship among the 19 lethals averaged 26 percent and their inbreeding coefficients averaged 11.7 percent.

cumference of 25 inches. These values exceed the average of the lethals reported here. The abnormal calf with the 241-day gestation weighed only 26 pounds and had a chest circumference of 19.5 inches. Even by discounting a little for possible breed differences, this comparison emphasizes the greatly reduced size of these hydrocephalic calves.

Limited information has been obtained on the degree of hydrocephalus (amount of fluid in the cerebral ventricles) and on the volume of the cranial cavity. Five abnormal calves from which this information was obtained had a mean cranial cavity volume of 284 cc. (range 235-321) and an average of 78 cc. (range 40-113) of fluid in the lateral ventricles. Another calf from which the photos in Figure 14 were made was one of the most extreme cases encountered. The volume of its cranial cavity was approximately 800 cc. but no record of the amount of cerebrospinal fluid was obtained.

An estimate of the volume of the brain tissue was determined by the difference between volume of cranial cavity and degree of hydrocephalus. This difference for the five calves mentioned above was 206 cc. Rusoff[6] reported brain weights of four normal, newborn calves; the average of these weights was 188 grams. The volume of the cranial cavity of three normal calves in this herd was 197 cc., and the amount of fluid in the ventricles was approximately five cc. Thus, the total amount of brain tissue in these lethals was apparently near normal.

Excessive amniotic fluid accompanied the birth of a few, but not all, of these hydrocephalic calves. This is shown by the photograph in Figure 16. The cow pictured had just given birth to an abnormal calf. Her subsequent reproductive history indicated that her reproductive system had not been affected. The excessive amniotic fluid observed in these cases was assumed to be associated with the lethal fetus. However, information regarding the nature of this condition and its relationship to the lethal is lacking.

Herd History and Genetic Aspects of the Lethal

The experimental beef cattle at the New Mexico Agricultural Experiment Station consist of a herd of purebred Herefords managed under farm conditions and a related herd of grade Herefords maintained under range conditions. The purebred herd was established in 1932. Since that time all bulls used for breeding have been produced in the herd except for one (Bull A) brought into the herd in 1940. No females have been introduced. Bull A sired 10 purebred offspring of which only six, four cows and two bulls, produced offspring in the herd. Bull D, a son of Bull A born in 1941, was used extensively in both the purebred and grade herds until 1951. By that time most of the younger animals in the herd were related to Bull A principally through the influence of Bull D. Bulls produced in the purebred herd have been used for breeding in the grade herd almost exclusively since 1935. The number of females of producing age in this herd has been near 150 during the period since 1940. The number of breeding females in the purebred herd has increased from 15 in 1940 to near 60 in 1957.

Nineteen abnormal calves have been recognized. Lethals resulted only when Bull A was a common ancestor on both sides of the pedigree. When it became apparent that the lethal traced to Bull A, those animals in the herd not related to Bull A were set aside as a separate line and are referred to as the "old line." Cattle related to Bull A made up a second line referred to as the "outcross" line.

Affected male and female calves occurred

in nearly equal numbers, indicating that the anomaly was not related to or influenced by the sex of the individual. Abnormal calves were produced in both the range and purebred herds under conditions of nutrition that varied from satisfactory for the purebred herd to very poor in some years for the grade herd. Environmental differences existing between these two herds, therefore, did not account for the lethal condition.

The facts suggest a single autosomal recessive gene in the homozygous state as being the genetic explanation for this lethal. Since no lethals have appeared in the old line after having been closed for over 25 years coupled with the fact that many matings of heterozygotes to old line cattle have never produced hydrocephalic calves is convincing evidence that the gene is not present in the old line.

A complicated pedigree chart showing all animals involved in each of the 19 lethal births would seem to contribute little to understanding the inheritance of this anomaly. Therefore, a partial pedigree showing the path by which the gene was introduced into the herd, the primary paths of dispersion throughout the herd, and typical matings that produced lethals is presented in Figure 17. Three of the six offspring of bull A that became parents in this herd have been identified as carriers of the lethal gene. This is sufficient evidence that bull A was heterozygous for this gene. Bull D sired an abnormal calf in a sire $\times$ daughter mating and he was the sire of several animals that proved to be carriers of the lethal gene. Bull C was never mated to outcross females but one of his daughters, cow E, transmitted the gene to at least three offsprings, one a lethal. The third offspring of bull A identified as a carrier, cow B, was of minor importance since she was not known to have produced a lethal and her heterozygous son was used only in the grade herd.

Since no matings were made specifically to test the mode of inheritance (i.e. mating of known carriers after their heterozygosity was discovered) the information obtained from the incidence of lethal calves resulting from the matings of heterozygous bulls to daughters of heterozygous bulls is the best available test of the proposed mode of inheritance. One hundred fifty-four such matings were counted. From them 19 lethals resulted. This tabulation included six sire $\times$ daughter matings that produced one lethal. The result (12.3 percent lethals) is in very close agreement with that expected (12.5 percent) from this type of mating and the proposed mode of inheritance. All daughters of heterozygous sires that produced calves when mated to heterozygous bulls were included in the tabulation. Since the daughters were selected to remain in the herd largely for reasons other than the probability of being heterozygous, these results should be relatively unbiased.

Summary

A lethal observed in an experimental herd of Hereford cattle was studied to obtain a description of the condition and to ascertain the mode of inheritance. Affected calves were dead at birth or died soon thereafter. Internal hydrocephalus, small body size, partial occlusion of the supraorbital foramen, small cerebellum, dome-shaped skull, and incompletely developed teeth characterized the lethal. The incompletely developed teeth and small size indicated prematurity, but most calves were only a few days premature. A notable exception was one calf with a 241-day gestation. The amount of cerebrospinal fluid found in the lateral ventricles ranges from 40 to 113 cc. The volume of brain tissue observed, approximately 200 cc., was considered normal. Body measurements indicated that the axial skeleton was more adversely affected than the appendicular skeleton. A few births were accompanied by excessively large amounts of amniotic fluid, a condition which appeared to be associated with the lethal fetus.

Evidence from the herd history, the types of matings that produced lethals and incidence with which the lethals appeared support the hypothesis of a single autosomal recessive gene being a satisfactory explanation of the inheritance of the lethal condition. The source of the recessive gene was traced to an outcross made in 1940. Except for this outcross the herd had been closed since 1932.

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